

GE Healthcare

ÄKTAcrossflow

User Manual



ÄKTA™

Important user information

All users must read this entire manual to fully understand the safe use of ÄKTAcrossflow.

WARNING!



The WARNING! sign highlights instructions that must be followed to avoid personal injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION!

The Caution! sign highlights instructions that must be followed to avoid damage to the product or other equipment. It is important not to proceed until all stated conditions are met and clearly understood.

Note

The Note sign is used to indicate information important for trouble-free and optimal use of the product.

Declaration of conformity

This product meets the requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a laboratory device. It is not intended for clinical or in vitro use, or for diagnostic purposes.
- used as a stand-alone unit, or
- connected to other CE-marked GE Healthcare instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

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1 Introduction

1.1 The User Manual

The ÄKTAcrossflow™ User Manual provides instructions for performing cross flow filtration, also called tangential flow filtration, using the automatic ÄKTAcrossflow instrument and UNICORN™ software.

1.2 Introduction

The purification of biological therapeutics requires processes which usually employ two technologies used in combination: membrane separations and chromatography. Chromatography offers greater selectivity than membrane separations, and can purify much greater quantities of solution in one operation.

However, membrane separations technologies offer a number of other advantages including the fact that they are a fast and robust process step.

ÄKTAcrossflow covers three main filtration techniques:

- Ultrafiltration of proteins
- Diafiltration of proteins
- Microfiltration of cell and lysate solutions

The system supports both hollow fibers and flat sheet cassettes with sizes suitable for flow rates up to 300 ml/min for flat sheet cassettes and up to 600 ml/min for hollow fibers.

The main application is method development and scale-up in process development laboratories.



WARNING! ÄKTAcrossflow is intended for laboratory use only, not for clinical or *in vitro* diagnostic purposes.

1.3 Glossary of terms

Term	Explanation
Cartridge	Also Module or Cassette. The unit encapsulating the membrane. The membrane can have different format.
Flat sheet cassette	Example of the unit encapsulating the membrane. A cassette has at least one inlet (feed) and two outlets (retentate and permeate).
Hollow fiber	The tube-like structure made from a membrane and sealed inside a cross flow cartridge.
Cross flow	Also called tangential flow filtration. In cross flow filtration, the feed solution flows parallel to the surface of the membrane. Driven by pressure, some of the feed solution passes through the membrane filter. Most of the solution is circulated back to the feed tank. The movement of the feed solution across the membrane surface helps to remove the buildup of foulants on the surface.
Cut-off	MWCO, Molecular weight cutoff. The size designation in Daltons (D) for ultrafiltration membranes. The molecular weight of the globular protein that is 90% retained by the membrane. No industry standard exists; hence the MWCO ratings of different manufacturers are not always comparable.
Retentate	The portion of the feed solution that does not pass through a cross flow membrane filter.
Permeate	Also called filtrate. The portion of a process fluid that passes through a membrane.

1.4 Principles

1.4.1 Normal flow filtration versus cross flow filtration

GE Healthcare ÄKTAcrossflow, separation cartridges, flat sheet cassettes and hollow fibres, are designed for cross flow (tangential flow (TFF)) operation. Unlike normal flow filtration, or dead-ended filtration, cross flow methodology continuously sweeps the membrane surface by recirculating the feed flow across it. Doing so minimizes blinding of the membrane and promotes consistent, long-term productivity. It also allows units to be cleaned, stored, and re-used as needed.

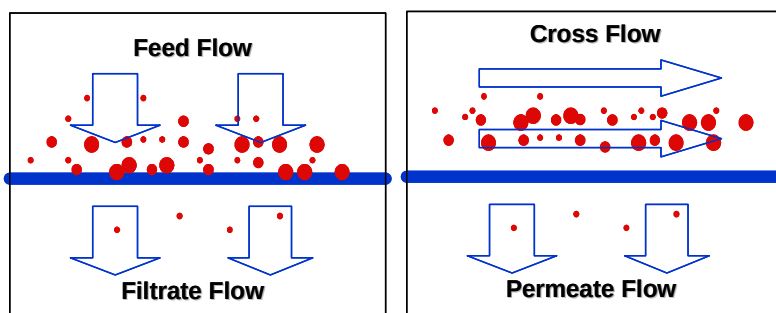


Fig 1-1. Normal flow and tangential flow filtration.

1.4.2 System flows

As the feed flow is pumped through the cartridge the retentate, the material excluded by the membrane pores, continues through the recirculation loop. The permeate, including solvent and solutes, is transported through the membrane pores and is collected separately.

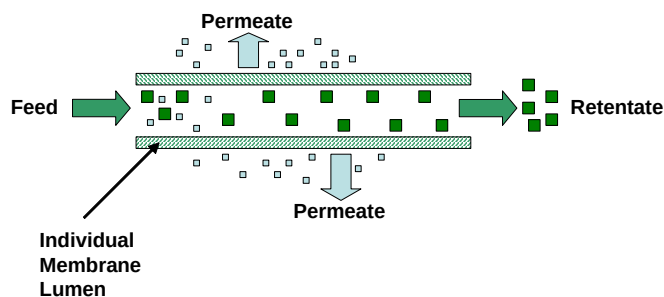


Fig 1-2. Principle of cross flow filtration.

1.4.3 Filtration effects

Term	Definition
Q_F, Q_R, Q_P	Feed, retentate and permeate flow respectively [ml/min]
P_F, P_R, P_P	Feed, retentate and permeate pressure respectively [Bar]

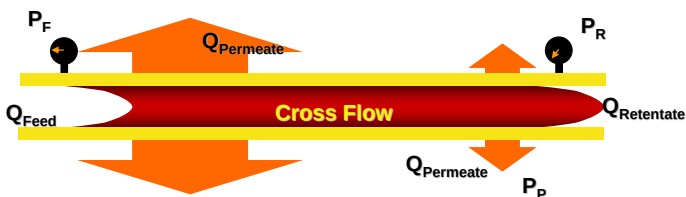
- The filtration effect in cross flow filtration is a result of the applied “transmembrane pressure TMP”.

$$TMP = \frac{P_F + P_R}{2} - P_P$$

- DeltaP (ΔP) is the pressure drop between feed and retentate:

$$\Delta P = P_F - P_R$$

- Cross flow is proportional to DeltaP, if permeate flow is zero.



The workflow of a cross flow system can be illustrated simplified as below.

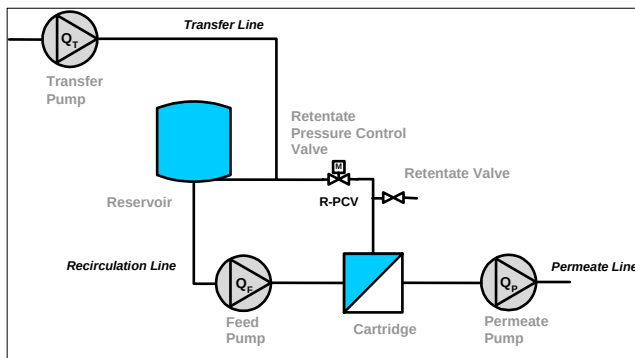


Fig 1-3. A cross flow system, simple illustration.

1.5 ÄKTAcrossflow applications

1.5.1 Ultrafiltration

Typical cut-offs: 1 kD to 1000 kD.

Ultrafiltration is a pressure driven, convective process using semi-permeable membranes to separate species by molecular size or shape. Removing solvent from solution results in solute concentration or enrichment. In ultrafiltration, species smaller than the membranes pores pass through the membrane while larger species are retained.

UF membranes may also be used for diafiltration to remove salts or other microspecies from a solution via continuous dilution and re-concentration.

Protein concentration

The volume of sample in the reservoir is reduced. The product is retained at the retentate side of the membrane.

If the sample volume is larger than the reservoir volume, the reservoir can be continuously fed with sample solution (Fed Batch).

Ultra filtration	
Retentate	Proteins
Membrane	
Permeate	Small peptides and salts.
Cut-off	1 kD to 1000 kD.

Diafiltration

Diafiltration is a unit operation that incorporates ultrafiltration membranes to remove salts or other microsolute from a solution. Small molecules are separated from a solution while retaining larger molecules in the retentate.

Buffer exchange is a filtration process used for the removal of smaller ionic solutes, whereby the feed solution is continuously, or repeatedly, filled up with a buffer. One buffer is removed and replaced with an alternative buffer.

A buffer exchange is typically run after an UF concentrating step using the same cassette/hollow fiber as for the UF step. Again, the product is retained at the retentate side, in the reservoir. Typical cut-offs are 1 kD to 1000 kD.

Ultra filtration

Retentate	Proteins (in new buffer).
Membrane	
Permeate	Small peptides and salts (old buffer).
Cut-off	1 kD to 1000 kD.

1.5.2 Microfiltration

Microfiltration is a pressure driven convective process, intended to separate larger insoluble particles (sub-micron size species) resulting in solution concentration or clarification.

It is usually an upstream recovery process where cells and cell debris are separated from the other components in the solution. The product can be the cells in the retentate or the clarified protein solution in the permeate.

Typical cut-offs are 100 nm to 10 μm .

Micro filtration	
Retentate	Intact cells, cell debris.
Membrane	
Permeate	Colloidal material, viruses, proteins and salts.
Cutoff	100 nm to 10 μm .

Cell harvesting

This may be the process of concentrating, dewatering, the cell mass after fermentation. With cell harvesting, the cells are the target material, and are recovered as product in the retentate.

Cell washing

The cells may also be washed to prepare them, e.g transfer them into a specific buffer for further processing, such as freezing or lysing. Together with cell harvesting, a cell washing step can be performed.

Lysate clarification

Cells, cell debris or other insoluble matter are retained by the membrane and the target product is passing through the membrane into the permeate. Together with lysate clarification, a diafiltration step can be performed to maximize target product recovery from the lysate.

1.6 The ÄKTAcrossflow

1.6.1 Description

In ÄKTAcrossflow, the sample solution is loaded into the Reservoir with the Transfer pump. The Feed pump transports the solution to the filtration device. The flow through the membrane is regulated by adjusting the pressure using the Retentate pressure control valve (R-PCV). The remaining retentate flow, cross the membrane, is recirculated back to the Reservoir. In diafiltration, the Transfer pump is transporting solution to the reservoir during the process in order to maintain a constant retentate volume.

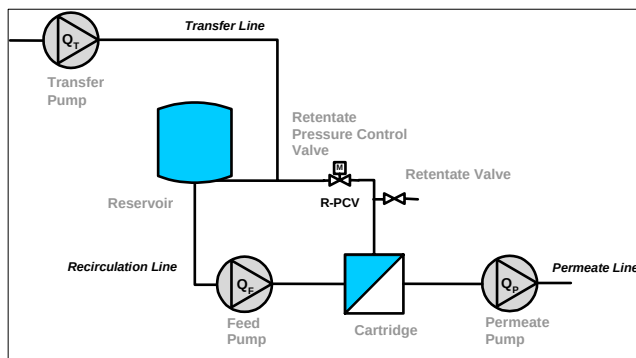


Fig 1-4. Flow path: Overview.



Fig 1-5. ÄKTAcrossflow system.

1.6.2 Control modes

Term definitions

Term	Definition
Q_F, Q_R, Q_P	Feed, retentate and permeate flow respectively [ml/min]
P_F, P_R, P_P	Feed, retentate and permeate pressure respectively [bar]
TMP	Transmembrane pressure[bar]: $TMP = \frac{P_F + P_R}{2} - P_P$
DeltaP	Pressure drop [bar]: $\Delta P = P_F - P_R$
Flux [$l/(h \cdot m^2)$]	Permeate flowrate per membrane area: [$l/(h \cdot m^2)$] Q_P = Permeate flow [l/h] A = Membrane area [m^2] $Flux = \frac{Q_P}{A}$
Shear rate [sec^{-1}]	Q_f = Feed flow (ml/min) n = Number of fibers r = Fiber radius [mm] Shear = $169.76527 \times Q_f / n \times r^3$

TMP Control mode

Control Mode: TMP Control	Control Element:		
	Feed Pump	Permeate Pump	R-PCV
TMP control with constant Feed flowrate	$Q_F > 0$	Offset	TMP
TMP control with constant Retentate flowrate	$Q_R > 0$	Offset	TMP
TMP control with constant DeltaP	$P_F - P_R > 0$	Offset	TMP

TMP control is usually used in ultrafiltration where the system has to push the retentate through the relatively small pores of the membrane. The control mode is used at α :

- Constant Feed flow,
- Constant Retentate flow, or
- Constant DeltaP.

The TMP is mainly controlled by the Retentate valve (R-PCV). The TMP control mode adjust the Retentate control valve and permeate pump to maintain set TMP. Before activation of TMP control, DeltaP has to be stable.

Offset is 0.2 bar, as default, and is used to avoid low or negative pressure on the permeate side. Too low pressures will affect the permeate pump's function as a flow meter.

If $TMP < \Delta P/2$, the Permeate pump Offset will automatically be changed.

The TMP control mode resets Flux control and Permeate Unrestricted flow.

Note: *The TMP has to be optimized for different applications (filter and sample). See Chapter 6.*

Flux Control mode

Control Mode: Flux Control	Control Element:		
	Feed Pump	Permeate Pump	R-PCV
Flux control with constant Feed flowrate	$Q_F > 0$	Flux > 0	Offset, Unrestricted for $P_p > \text{Offset}$
Flux control with constant Retentate flowrate	$Q_R > 0$	Flux > 0	Offset, Unrestricted for $P_p > \text{Offset}$
Flux control with constant Shear rate	shear rate > 0	Flux > 0	Offset, Unrestricted for $P_p > \text{Offset}$
Flux control with constant DeltaP	$P_F - P_R > 0$	Flux > 0	Offset, Unrestricted for $P_p > \text{Offset}$

Flux control is usually used in microfiltration filtration where the system has to hold back the permeate from the relatively large pores of the membrane. The control mode is used at a:

- Constant Feed flow, or
- Constant Retentate flow, or
- Constant Shear rate, or
- Constant DeltaP.

In this mode, the TMP value is a function of the permeate flux.

If the permeate pressure is <0.2, the R-PCV has to increase the Retentate pressure. When permeate pressure is above 0.2, the permeate pump can start. A constant ramping during 60 sec. from flux 0 to the set flux is performed. During the ramping, the function for R-PCV is to maintain the permeate pressure above 0.2.

Note: If the system reaches TMP limit when active, the system will Pause.

Flux control resets TMP control, and Permeate Unrestricted flow.

Note: The flux has to be optimized for different applications (filter and sample).

Permeate Unrestricted flow

Control Mode: Permeate Unrestricted flow	Control Element:		
	Feed Pump	Permeate Pump	R-PCV
With constant Feed flow rate	$Q_F > 0$	$P_R = P_P$ ($\geq$ Offset)	$P_R = P_P$
With constant Retentate flow rate	$Q_R > 0$	$P_R = P_P$ ($\geq$ Offset)	$P_R = P_P$
With constant Shear rate	Shear rate > 0	$P_R = P_P$ ($\geq$ Offset)	$P_R = P_P$
With constant DeltaP	$P_F - P_R > 0$	$P_R = P_P$ ($\geq$ Offset)	$P_R = P_P$

Permeate unrestricted flow starts the flow on the permeate pump at Offset permeate pressure (0.2 bar default). If Retentate pressure $>$ Offset, the Retentate pressure value is used as a new Offset. If Retentate pressures $<$ Offset, the Retentate control valve lifts Retentate pressure to Offset.

Permeate unrestricted flow resets TMP control and Flux control.

The mode is used, for example, in the Method Wizard created preproduct method "Rinsing", see Section 5.2.1.

Normal Flow Filtration

In the Normal Flow Filtration mode, the flow and pressure over the filter are controlled by the feed pump only. In the permeate line, the permeate control valve (P-PCV) is fully opened. As a consequence, liquid can be pushed through the permeate pump as the check valves of the permeate pump are not active. In order to allow for a homogeneous flushing of the pump heads of the permeate pump during Normal Flow Filtration, the permeate pump is running idle at a low flow rate (20% of Feed flow) while liquid is transferred by the feed pump.

The pressure over the NFF filter (pNFF) is determined as the difference between feed pressure and permeate pressure in order to compensate for a minor pressure loss in the components of the permeate line:

$$pNFF = \text{feed pressure} - \text{permeate pressure}$$

Control Mode: Normal flow filtration	Control Element:		
	Feed Pump	Permeate Pump	PCV
NFF control with constant Feed flow	100%	20% of Feed flow)	R-PCV: Closed P-PCV: Opened
NFF control with constant Pressure	Set point fulfilled (pNFF)	20% of Feed flow)	R-PCV: Closed P-PCV: Opened

Normal Flow Filtration is used in Water flux test for hollow fibers with cut off 0.1 µm and larger

1.6.3 Sensors and valves

The system consists of several valves and sensors as shown in Fig. 1-6. There are sensors for:

- Pressure,
- Conductivity,
- UV absorbance,
- pH,
- Temperature,
- Reservoir level, and
- Air.

The sensors and valves are described in *ÄKTAcrossflow Instrument Handbook*.

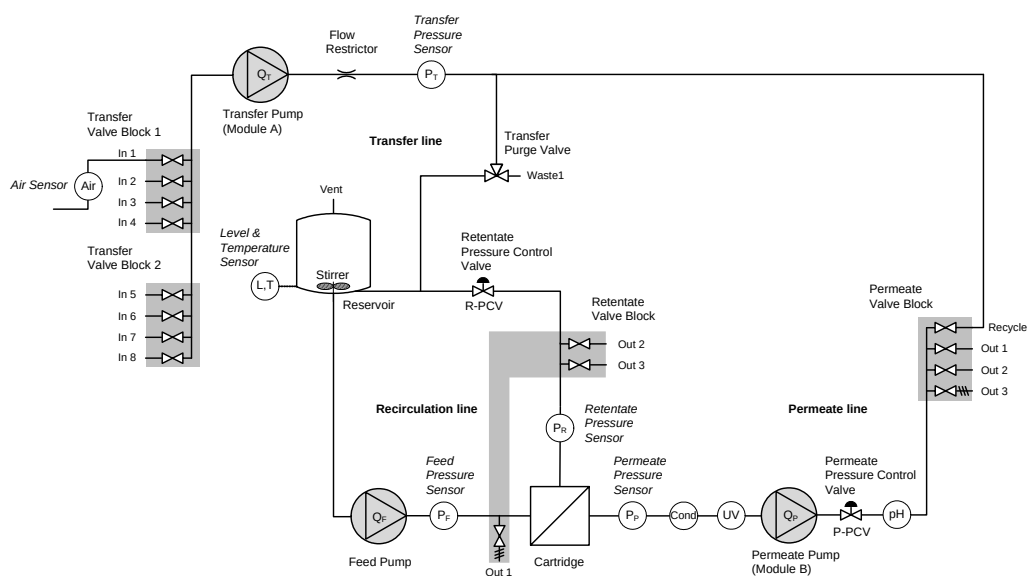


Fig 1-6. Detailed flow path

The output from the sensors are monitored during the process run with UNICORN.

1.7 Filtration cartridges

1.7.1 Kwick Start™ flat sheet cassettes

The cassettes are available in 50 cm² for ultrafiltration process development with minimum working volume.

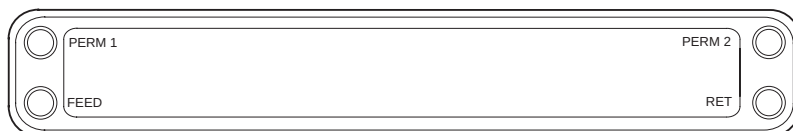


Fig 1-7. Ultrafiltration cassette.

Name:	Kwick Start
Cut-offs:	5kD, 10kD Select, 10kD, 30kD, 50kD and 100kD
Nominal area:	50 cm ²
Feed flow rate 300-480 LMH:*	25 - 40 ml/min
Flux range, low = 15 LMH:*	1.2 ml/min
Flux range, high = 150 LMH:*	12.0 ml/min
30 - 200 L/m ² vol challenge:*	135 -900 ml

*) The ranges relate to NMWC and are typical, not absolute values.

Table 1-1. Flat sheet cartridges with UNF fittings.

For ordering information, see *ÄKTAcrossflow Instrument Handbook*.

1.7.2 Hollow fibers

GE Healthcare manufactures a complete selection of cross flow ultrafiltration and microfiltration membranes with a surface area of 50 cm² specially dedicated for the ÄKTAcrossflow instrument. These designs are configured to both afford convenient linear scaling and to optimize any candidate application around reproducible and predictable fluid mechanics.

The tube-like structure is made from a membrane and sealed inside a cross flow cartridge. When in use, the feed stream flows into the inner diameter of one end of the hollow fiber and the retentate (the material that does not permeate through the walls of the hollow fiber) flows out the other end. The material that passes through the membrane (walls of the hollow fiber) is called the permeate.



Fig 1-8. Hollow fiber.

For ÄKTAcrossflow following hollow fibers are recommended:

Name	Cutoff	Inner diameter [mm]	Length [cm]	# fibers	Nominal Area [cm ²]	Feed flow rate 2000-16000 s ⁻¹	Flux range low = 10 LMH	Flux range high = 80 LMH	30-200l/m ² vol challenge
Start AXH	3 kD	0.5	60	4	40	10-85 ml/min	0.7 ml/min	5.6 ml/min	0.13-0.84 L
Start AXH	10 kD	0.5	60	4	40	10-85 ml/min	0.7 ml/min	5.6 ml/min	0.13-0.84 L
Start AXH	30 kD	0.5	60	4	40	10-85 ml/min	0.7 ml/min	5.6 ml/min	0.13-0.84 L
Start AXH	100 kD	0.5	60	4	40	10-85 ml/min	0.7 ml/min	5.6 ml/min	0.13-0.84 L
Start AXH	300 kD	0.5	60	4	40	10-85 ml/min	0.7 ml/min	5.6 ml/min	0.13-0.84 L
Start AXH	500 kD	0.5	60	4	40	10-85 ml/min	0.7 ml/min	5.6 ml/min	0.13-0.84 L
Start AXM	500 kD	1.0	30	6	50	70-560 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	750 kD	1.0	30	6	50	70-560 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	3 kD	0.5	30	12	50	24-200 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	10 kD	0.5	30	12	50	24-200 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	30 kD	0.5	30	12	50	24-200 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	100 kD	0.5	30	12	50	24-200 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	300 kD	0.5	30	12	50	24-200 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	500 kD	0.5	30	12	50	24-200 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	0.1 µm	1.0	30	6	50	70-560 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	0.2 µm	1.0	30	6	50	70-560 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	0.45 µm	1.0	30	6	50	70-560 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L
Start AXM	0.65 µm	0.75	30	8	50	40-320 ml/min	0.8 ml/min	6.4 ml/min	0.15-1.0 L

Table 1-2. Hollow fiber cartridges with UNF fittings.

For assembling instructions and ordering information, see *ÄKTAcrossflow Instrument Handbook*.

1.8 Associated documentation

The following documentation is included with ÄKTAcrossflow. ÄKTAcrossflow manuals are delivered as spiral bound books or binders, as indicated in the margin.

Screen versions of the documentation are available from UNICORN software under **Help**.



1.8.1 ÄKTAcrossflow Installation Guide

This guide provides technical information and basic operating instructions for the unpacking and installation of ÄKTAcrossflow.



1.8.2 ÄKTAcrossflow Instrument Handbook

This handbook provides technical information and basic operating instructions for the ÄKTAcrossflow. In addition, maintenance schedules, instructions for troubleshooting and user maintenance are included.



1.8.3 ÄKTAcrossflow Safety Handbook

Provides the safety instructions that must be strictly followed for the safe use of the ÄKTAcrossflow.



1.8.4 ÄKTAcrossflow Method Handbook

Provides more detailed information on applications.

1.8.5 UNICORN documentation

UNICORN control system includes three manuals:



- *Getting Started*
- *User Reference Manual (2 pcs)*
- *Administration and Technical Manual*



Documentation of the ÄKTAcrossflow specific Evaluation module includes:

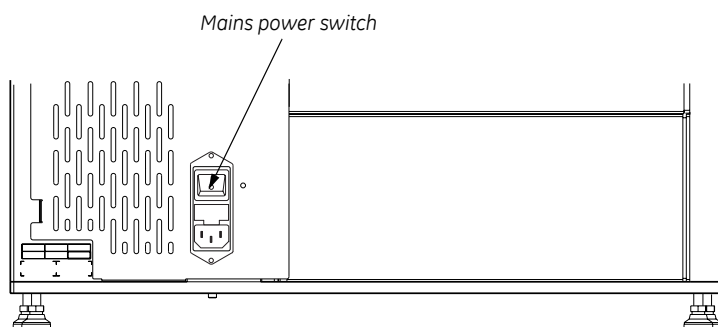
- *User Reference Manual - UNICORN 5.1 - Evaluation for Cross Flow Filtration*
- Specific sections in Online Help

2 System preparation

2.1 Starting the system

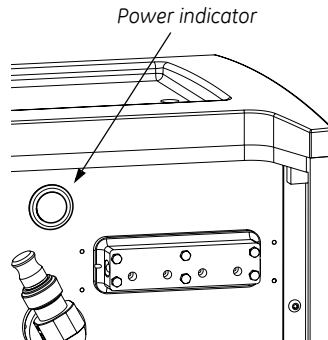
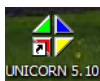
To start ÄKTAcrossflow system:

- 1 Switch on the instrument at the mains power switch located on the rear panel.



The **Power** indicator on the front panel flashes slowly until the internal communication with the CU (Control Unit) is established.

- 2 Switch on power to the PC and the monitor.
- 3 Start and log on to UNICORN by double-clicking on the icon on the Microsoft® Windows® XP desktop.

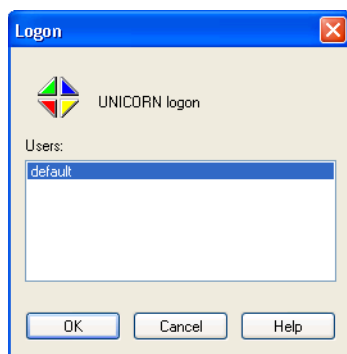


- 4 Enter **User name** and **Password** and click **OK**.

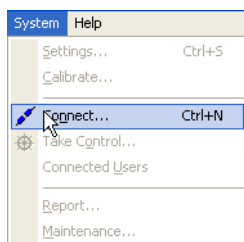


Normally the system administrator defines the users and creates your first password. The program can also be set up so that you can log on without a password. See UNICORN manuals and Online Help.

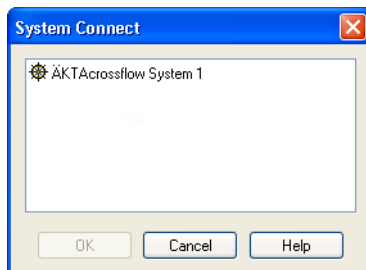
Or login as default user with password "default".



- 5 In the **System Control** module, select **System:Connect...** to connect UNICORN to the instrument unit.

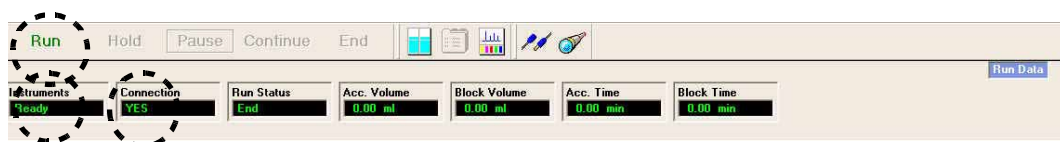


- 6 Select the appropriate system name and click **OK**.



When the communication between UNICORN and the instrument unit is established:

- There is a constant light on the **Power** indicator on the instrument unit.
- The green **Run** indicator in the status bar in UNICORN is lit.
- The **Connection** box shows **Yes**.
- The **Instrument** box shows **Ready**.



2.2 Assembling Filters

For preparing and connecting Flat sheet membrane cartridges or Hollow fiber membrane cartridges, see *ÄKTAcrossflow Instrument Handbook*.

2.3 The pump piston rinsing system

2.3.1 Introduction

Leakage between the pump chamber and the drive mechanism is prevented by a seal. The seal is continuously lubricated by the presence of eluent. To prevent any deposition of salts from aqueous buffers on the pistons and to prolong the life of the seals, the low pressure chamber behind the piston can be flushed continuously with a low flow of 10 mM NaOH in 20% ethanol prepared in Milli-Q™ water or equivalent.

The piston rinsing system tubing is connected to the rearmost holes on the pump heads. The following flow diagram and table show the tubing configuration of the piston rinsing system.

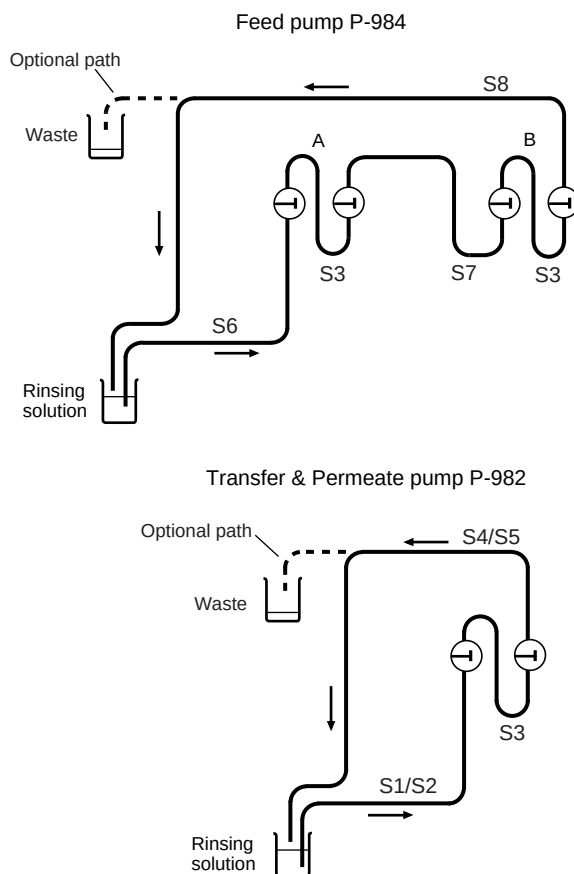


Fig 2-1. Piston rinsing system.

To eliminate the risk of re-introducing proteins/cells into subsequent batch runs, always use a separate waste container. Do not recirculate the same rinsing solution!

If rinsing solution is recirculated, the solution should be replaced with fresh solution every day or between cross flow runs.

To estimate the required volume of rinsing solution, the flow rate of rinsing solution is approx. 2/3 of the set flow of the pump.

In connection to System sanitization, the rinsing bottles should be cleaned.

2.3.2 Using the pump piston rinsing system

To use the piston rinsing system:

- 1 Fill the rinsing system bottles with 10 mM NaOH in 20% ethanol, see Fig. 2-2.
- 2 Insert the rinsing inlet and outlet tubing ends into the rinsing solution, see Fig. 2-1.

Note: *To eliminate the risk of re-introducing bacteria, etc., use the optional path with a separate waste bottle.*

- 3 Fill the tubing with solution using a syringe connected to the outlet tubing end.
- 4 Repeat the procedure for all pumps.

2.3.3 Cleaning the pump piston rinsing system

- 1 Remove the rinsing solution bottles and empty them. Place the tubing in waste containers.
- 2 Fill the bottles with freshly prepared rinsing solution, (20% ethanol in 10 mM NaOH). However, do not connect the rinsing system tubing yet.

Rinsing system bottle
Transfer & Permeate pump

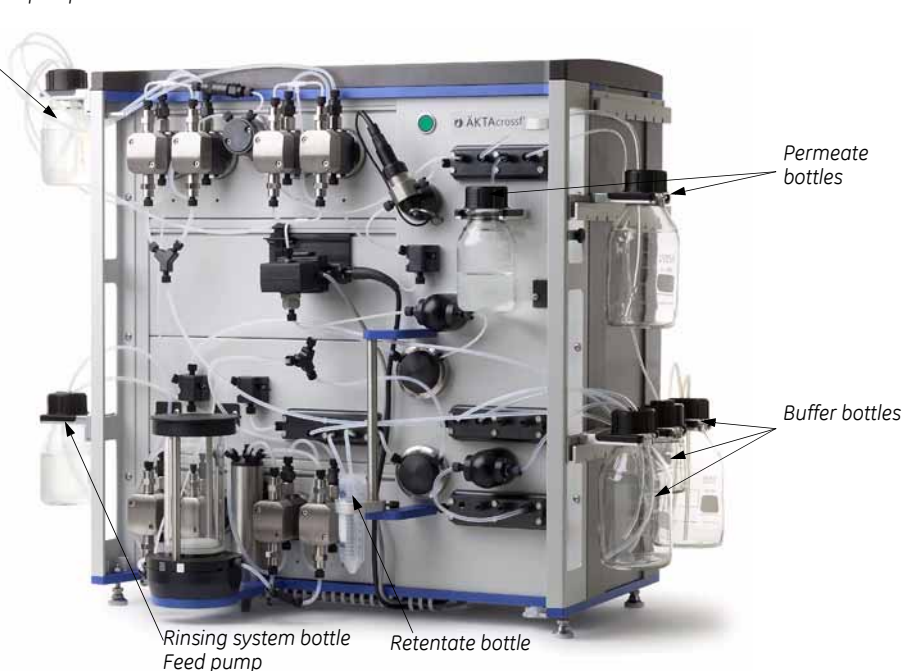
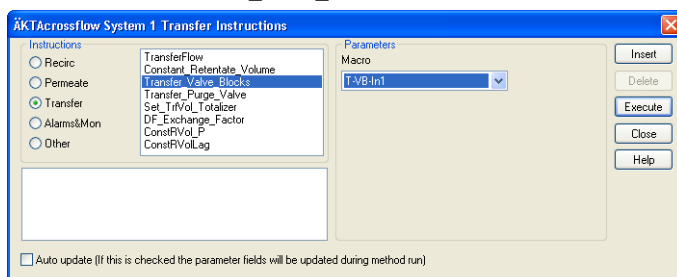
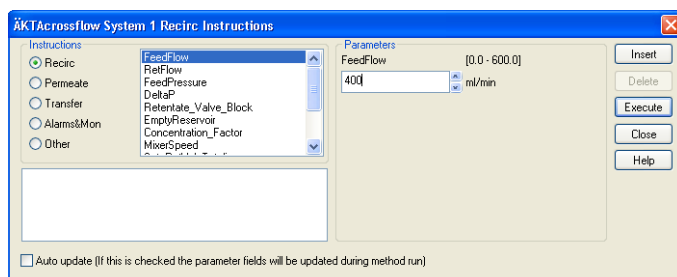
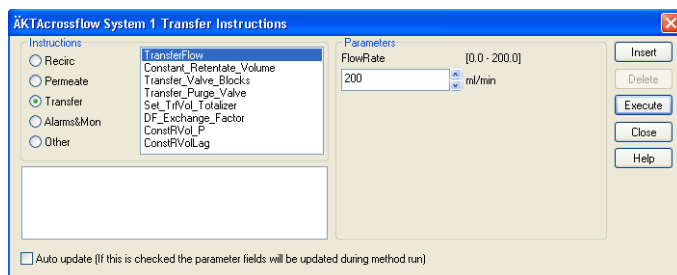


Fig 2-2. Location of the bottles.

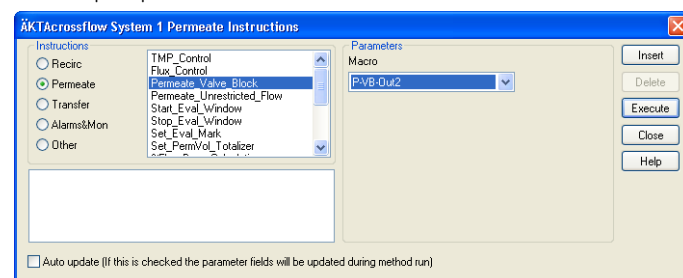
- 3 Connect one Transfer Inlet port to distilled water. In **Manual**, select this port under Transfer; Transfer_Valve_Block, click **Execute**.



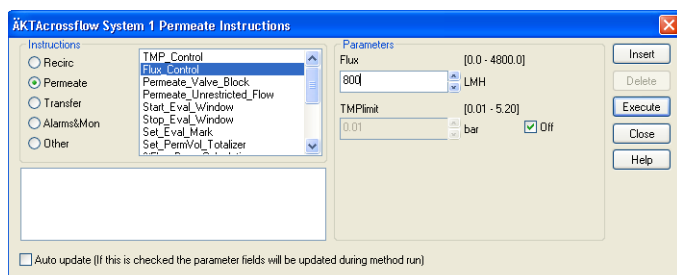
- 4 Start transfer pump at 200 ml/min and pump 200 ml distilled water to the reservoir. At the same time as the transfer flow is started, start a Feedflow at 400 ml/min.



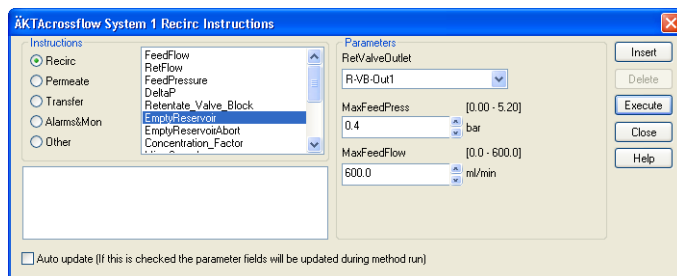
- 5 Open permeate valve block Out 2. When 200 ml water is in the reservoir, stop transfer pump.



- 6 Start Flux Control under Permeate, set flux to 800 LMH and pump for three minutes.



- 7 After three minutes, set Flux to 0 LMH and stop feed pump.
- 8 Select EmptyReservoir under Recirc, select R-VB-Out1 as RetValveOutlet, set MaxFeedPressure to 0.4 bar and click **Execute**.

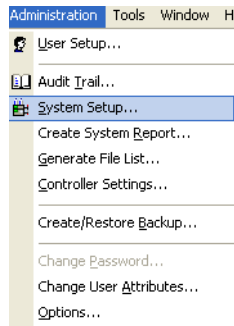


System will stop when reservoir is empty.

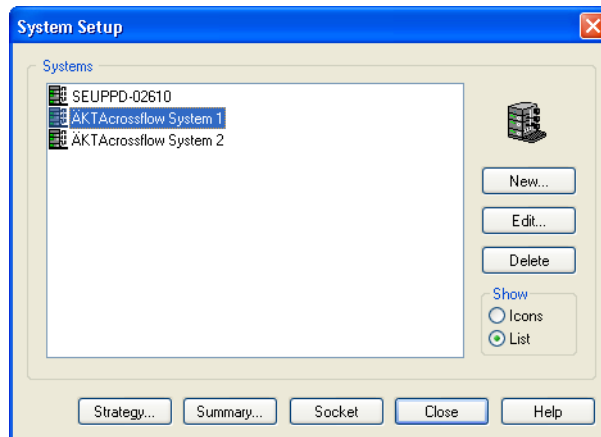
- 9 Immerge rinsing system tubing to rinsing solution bottles and repeat from the beginning (Step 3).

2.4 Selecting filter component for the system strategy

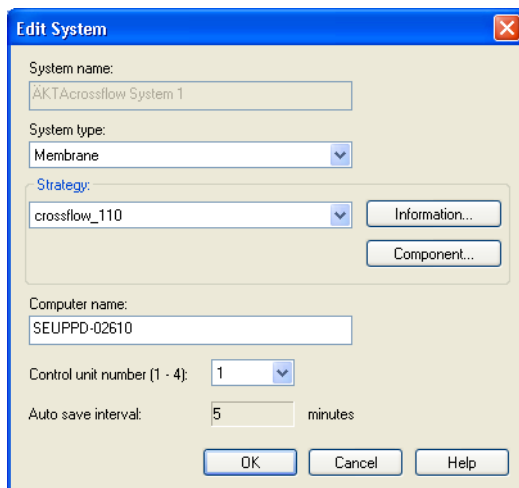
- 1 In **UNICORN Manager**, select **Administrator: System Setup**.



- 2 Select a system and click **Edit**.

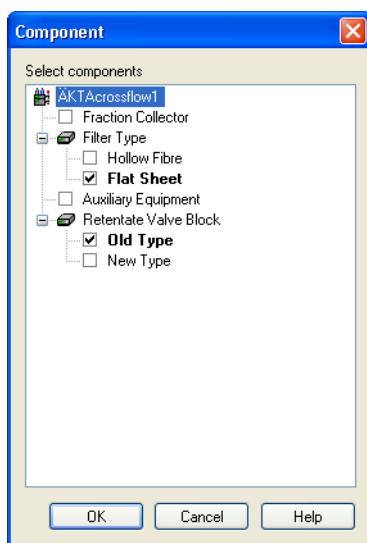


- 3 In the **Edit System** dialog, click **Component**.



- 4 In the **Component** dialog, select filter type:

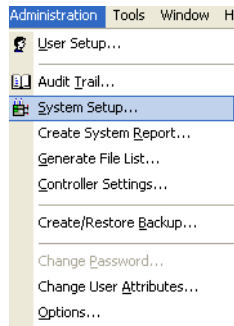
- Hollow fiber, or
- Flat Sheet.



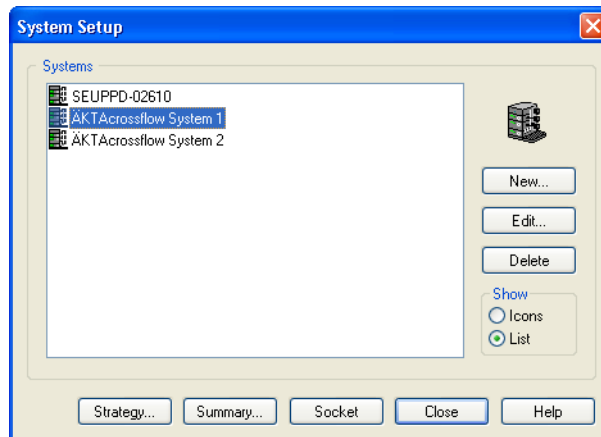
- 5 Click **OK** to close the **Component** dialog.
- 6 Click **OK** to close the **Edit System** dialog.
- 7 Click **Close** to close the **System Setup** dialog.

2.5 Selecting type of Retentate valve block

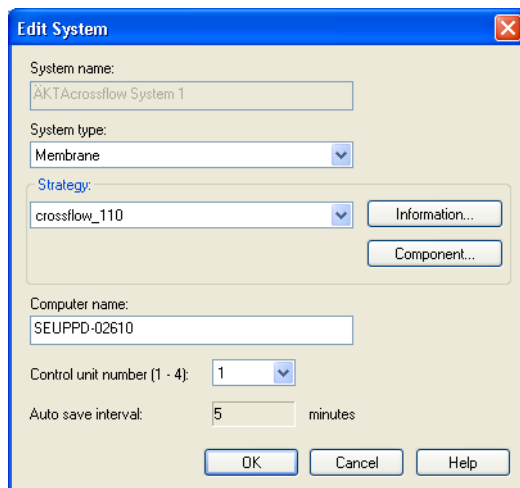
- 1 In **UNICORN Manager**, select **Administrator: System Setup**.



- 2 Select a system and click **Edit**.

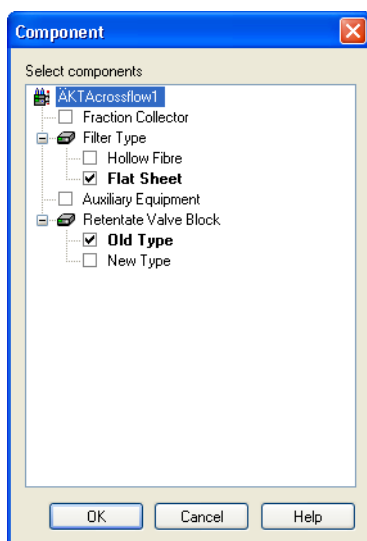


- 3 In the **Edit System** dialog, click **Component**.



- 4 In the **Component** dialog, select type of Retentate valve block used:

- Old type, or
- New type.



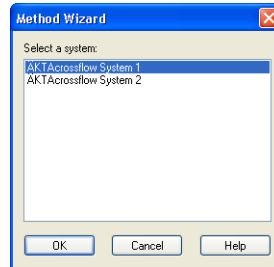
- 5 Click **OK** to close the **Component** dialog.
- 6 Click **OK** to close the **Edit System** dialog.
- 7 Click **Close** to close the **System Setup** dialog.

2.6 Calibrating the level sensor

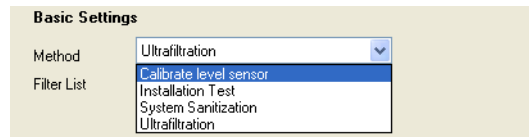
There is a method for automatic calibration of the level sensor available for the system. The calibration should be performed before every run.

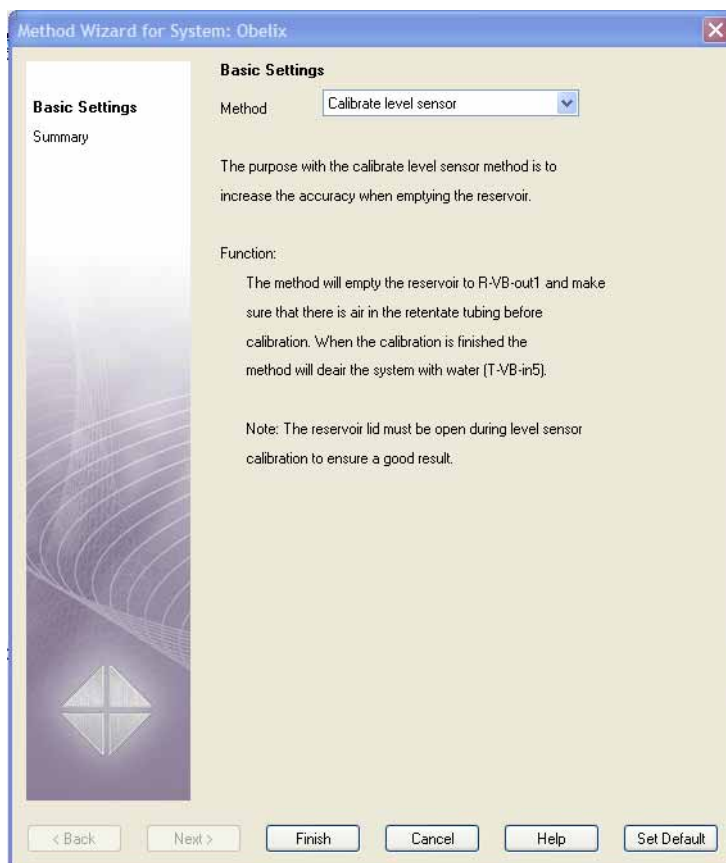


- 1 In the **Method Editor** of **UNICORN**, click the Method Wizard icon.
- 2 Select System.



- 3 In the **Basic Settings** dialog select Calibrate level sensor as Method.





- 4 Click **Finish**.
- 5 To run the method there are two alternatives:
 - Save the method and run it, see Section 9.2.
 - Run the method as an Instant run, see Section 9.3.

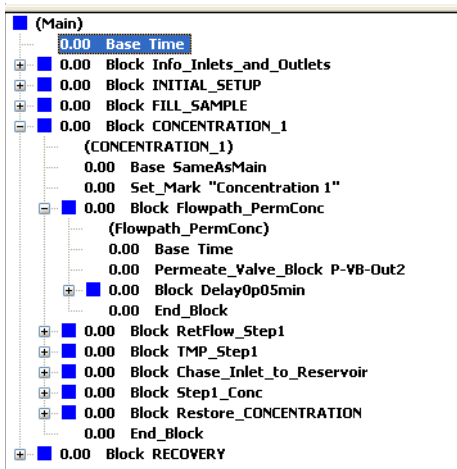
Note: If the level sensor is too far off the correct value, e.g. the system has not been used for some days, this method will not run properly. It is then necessary to manually make sure that the reservoir is empty and to perform a manual level sensor calibration in System Control. See *ÄKTAcrossflow Instrument Handbook*.

3 Handling methods in ÄKTAcrossflow

3.1 A UNICORN method

3.1.1 Blocks

The **Text pane** in the **Method Editor** of UNICORN displays the method as a list of text instructions. The instructions are usually organized in blocks, denoted by blue square symbols, for a specific functional use, for example to load a sample, to concentrate a sample etc. A block may contain other blocks or individual instructions. The blocks can be expanded to show the instructions within the block.



3.1.2 Base

Every method block must start with a **Base** instruction, defining the base for calculating breakpoints. Different blocks can use different bases. In ÄKTAcrossflow, the base can be one of the following:

- **Volume** (the unit depends on the scale defined in the system strategy)
- **Time** (minutes)
- **SameAsMain** (all blocks apart from the main block), which means that the block will inherit the base defined in the main block.

Note: Do not use **Column Volume (CV)** as base in ÄKTAcrossflow methods as it is not relevant and will lead to incorrect methods.

3.1.3 Calls

To execute the instructions contained within a block in a method, the block must be called by the program. When a block is called, the instructions in the block are executed in the order that they are written until the block is finished or the **End_Block** instruction is executed. There are two types of calls:

- Unconditional calls, which are made with a **Block** instruction.
- Conditional calls, which are made with a **Watch** instruction. This makes it possible to call a specified block or instruction when a particular monitor signal meets a given condition.

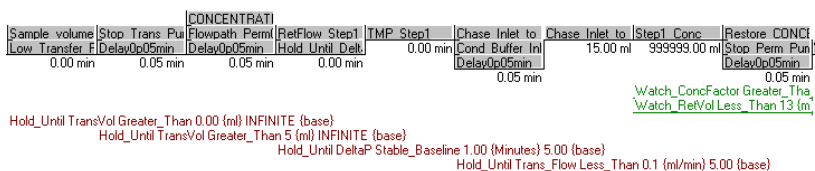
3.1.4 Watch and Hold_Until

The breakpoint when the **Watch** instruction is issued determines when the watch begins. A watch remains active until the condition is met or a new **Watch** instruction is issued for the same monitor. The watch is cancelled automatically when the condition is met. A watch can also be turned off with the **Watch_off** instruction.

The **Hold_Until** instruction is a special kind of **Watch** instruction. The method is put on hold until a specific condition is met (signal, test or value) or the time-out is reached. Thereafter the remaining instructions in the method are executed.

3.1.5 Block pane

The organization of blocks in the method is shown graphically in the **Block pane** of the **Method Editor**. Each block is represented by a gray bar with the block name and the length of the block. The line is shifted down to indicate calls to other blocks.



3.1.6 Run Setup

Run Setup in the **Method Editor** is a dialog box with a number of tabs that define the method properties.

Block	Variable	Value	Range
Start_Settings_System	Ret_Vol (ml)	13.00	0.00 - 5000.00
	Feed_Press_Alarm (bar)	5.20	0.00 - 5.20
	TMP_Alarm (bar)	5.20	0.00 - 5.20
Start_Settings_FS	Filter_Area (cm²)	50.0	1.0 - 1000.0
Sample_volume	Sample_Vol_High (ml)	0.00	0.00 - 80000.00
	Sample_Volume (ml)	5.00	0.00 - 80000.00
RetFlow_Step1	Ret_Flow_Step1 (ml/min)	25.0	0.0 - 600.0
Hold_Until_DeltaP	DeltaP (bar)	0.05	0.00 - 5.20
TMP_Step1	TMP_Step1 (bar)	2.00	0.00 - 5.20
Step1_Conc	Conc_Factor_Step1Cont	50.00	0.00 - 50.00
	Ret_vol_Step1Cont (ml)	13.00	0.00 - 80000.00
RetFlow_ProdRec	Ret_Flow_ProdRec (ml/min)	25.0	0.0 - 600.0
Ret_Flow	Ret_Flow (ml/min)	40.0	0.0 - 600.0

☐ Show details
☐ Show unused variables
☒ Display tooltip for extended variable cells

[Edit Variable...](#)
[Help](#)

3.2 Creating a new method

To create a new method, there are two alternatives:

- In the Method Wizard, customized methods for most purposes are made by setting appropriate values for the method variables.
- In the Text Instructions editor in the **Method Editor** module, more advanced editing facilities are available.

3.3 Creating cross flow methods using the Text instructions

The Text instruction editor in the Method Editor can be used to build methods step by step. The editor can also be used to modify instructions in methods created by the Method Wizard.

Note: All Strategy instructions for ÄKTAcrossflow are listed in Chapter 13.

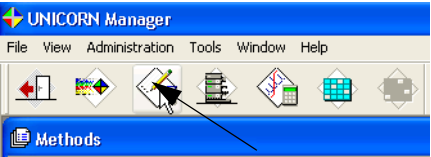
3.3.1 Opening the Text Instruction Editor

- 1 Select the **Method Editor** module in UNICORN.

If a method is opened in the **Method Editor**, click the Text Instruction icon.



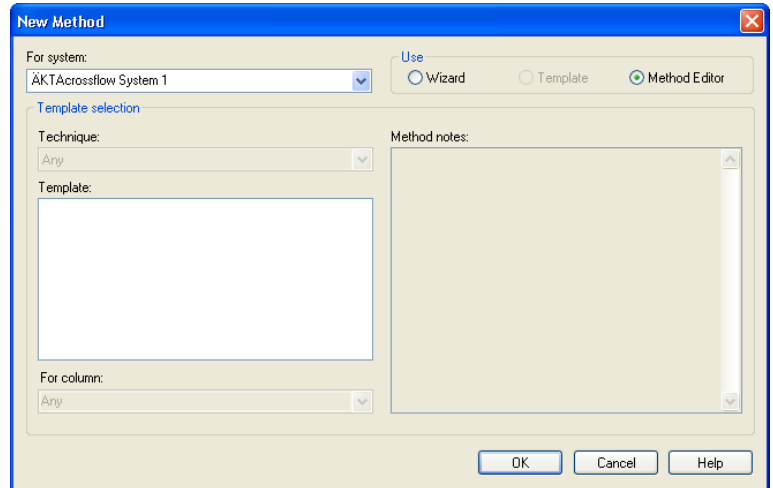
To create a new method, click the **New Method** icon in UNICORN Manager,



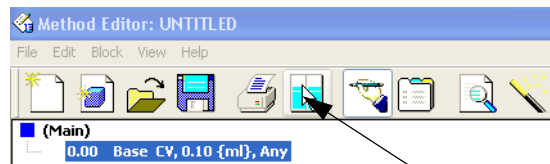
or click the **New** icon in the **Method Editor**.



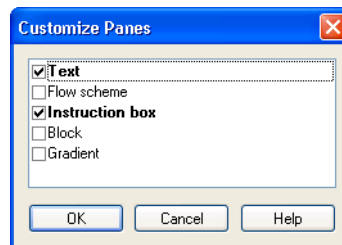
- 2 Select System and specify to use Method Editor. Click **OK**.



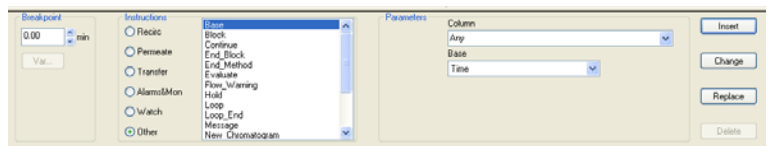
- 3 Click the **Customise Panes** icon.



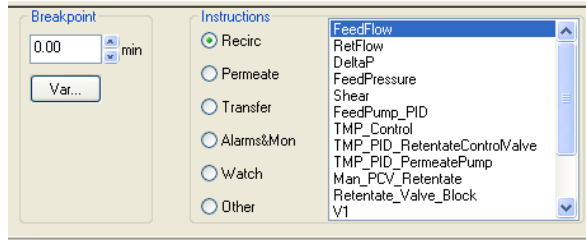
- 4 Select **Text** and **Instruction Box** and click **OK**.



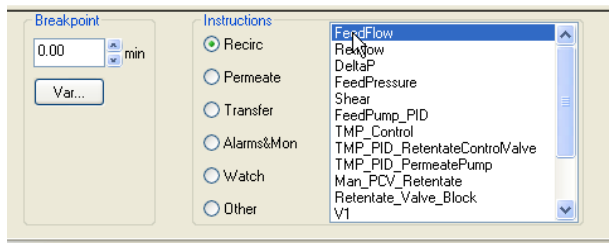
The Instruction box is displayed in the lower part of the **Method Editor**.



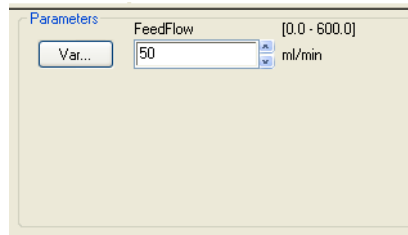
- 5 First select group of instructions, for example Recirc.



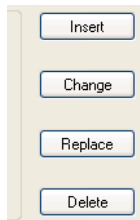
- 6 Select instruction within the group.



- 7 Enter a parameter value.



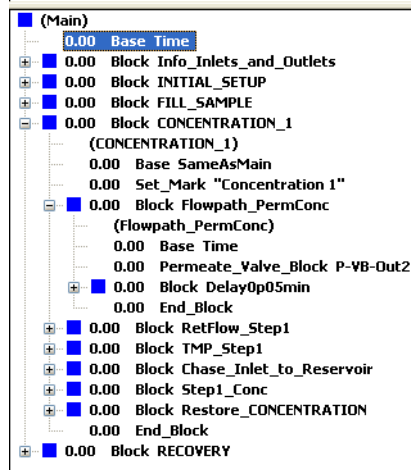
- 8 Use the **Insert**, **Change**, **Replace** or **Delete** buttons. All text entries are shown in the **Text** pane. Applicable variables can be edited for each selection.



- Click **Insert** to place the instruction in the method.
- To change a value in an instruction, select the instruction row, enter a new value and click **Change**.

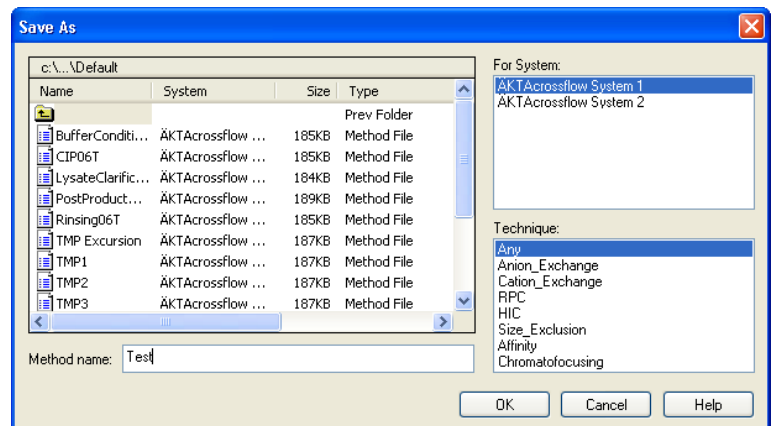
- To replace an instruction, select the row, edit the instruction and click **Replace**.
- To delete an instruction row, select the row and click **Delete**.

Individual text instructions can be grouped in blocks of instructions (marked by blue square symbols) for a specific functional use, for example to set a flow path, to rinse a filter. etc. A block may contain other blocks or individual instructions. This is an example of text instructions in the **Text** pane:



- To save the method, select **File:Save**.
- Select location.
- Enter method name, select System and Technique. Click **OK**.

Note: "Any" must be selected as Technique.



3.4 Example of creating an ÄKTAcrossflow method

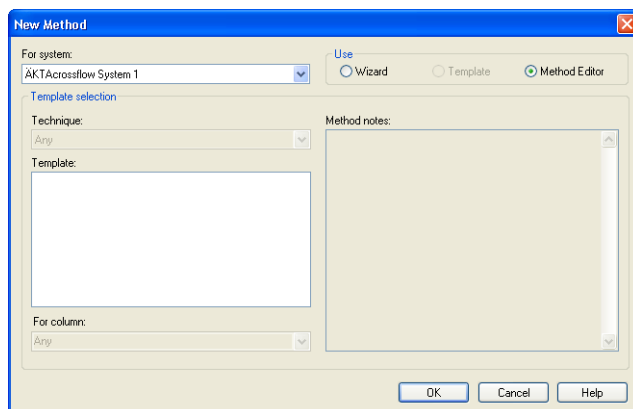
This is an example of a simple method which checks the function of the valves and pumps of ÄKTAcrossflow system.

```

(Main)
0.00 Base Time
0.00 Transfer_Valve_Blocks Closed
0.00 Transfer_Purge_Valve To_Reservoir
0.00 Permeate_Valve_Block Closed
0.50 Transfer_Valve_Blocks T-VB-In1
0.50 Permeate_Valve_Block P-VB-Recycle
0.50 Retentate_Valve_Block R-VB-Out1
1.00 Transfer_Valve_Blocks T-VB-In2
1.00 Transfer_Purge_Valve Waste
1.00 Permeate_Valve_Block P-VB-Out1
1.00 Retentate_Valve_Block R-VB-Out2
1.50 Transfer_Valve_Blocks T-VB-In3
1.50 Permeate_Valve_Block P-VB-Out2
1.50 Retentate_Valve_Block R-VB-Out3
2.00 Transfer_Valve_Blocks T-VB-In4
2.00 Permeate_Valve_Block P-VB-Out3
2.00 Retentate_Valve_Block R-VB-Out3
2.00 Transfer_Valve_Blocks T-VB-In5
2.00 TransferFlow 0.1 {ml/min}
2.50 Permeate_Valve_Block Closed
3.00 TransferFlow 0.0 {ml/min}
3.00 Transfer_Valve_Blocks T-VB-In6
3.00 FeedFlow 5.0 {ml/min}
3.00 Permeate_Unrestricted_Flow Enabled
3.00 Permeate_Valve_Block P-VB-Out2
3.50 FeedFlow 0.0 {ml/min}
3.50 Permeate_Valve_Block Closed
3.50 Transfer_Valve_Blocks T-VB-In7
4.00 Transfer_Valve_Blocks T-VB-In8
4.50 End_Method
  
```

Fig 3-1. Example of a simple ÄKTAcrossflow method.

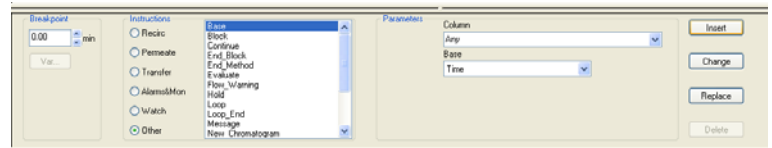
- 1 In the **Method Editor** of **UNICORN**, select **File: New**.
- 2 Select system, select to use Method Editor and click **OK**.



- 3 First the base of the method is selected. As default in UNICORN, column volumes (CV) is set as base. This is not useful in ÄKTAcrossflow methods.

(Main)
 0.00 Base CV, 0.10 {ml}, Any

- 4 In this case, "Time (minutes)" is more appropriate. Click the instruction row above, select Instruction group "Other", select Base, select Time as base and click **Change**.



The principle of inserting an instruction in a method is:

1) Enter the Breakpoint in minutes for the instruction

2) Select Instruction group

3) Select Instruction.

4) Select the command in the Macro list..

5) Click **Insert** to add the instruction to the method

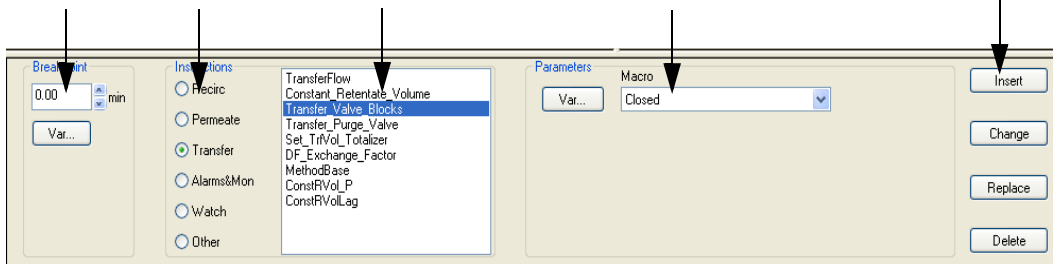
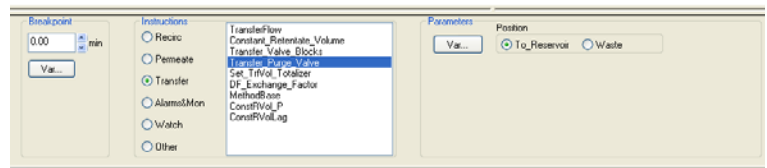


Fig 3-2. Principle of the instruction editor.

The instruction is inserted in the method.

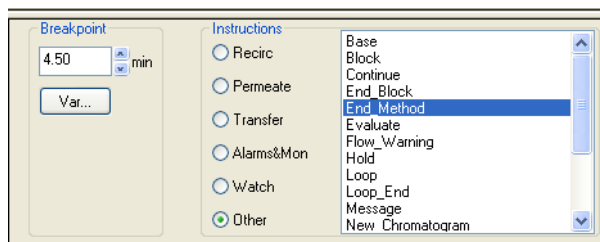
(Main)
 0.00 Base Time
 0.00 Transfer_Valve_Blocks Closed

- 5 Continue with the next instruction.

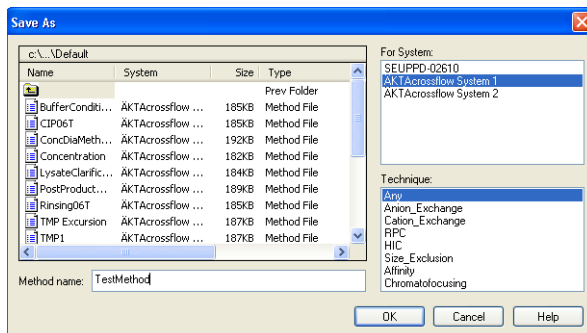


- 6 Repeat the procedure until all instructions are inserted in the method.

- To end the method, select Instruction group "Other" and the instruction End_Method.



- To save the method, select **File:Save**. Select System and Technique "Any". Enter a method name and click **Save**.



3.5 Creating cross flow methods using the Method Wizard

ÄKTAcrossflow methods are complicated and include many blocks and instructions. So, it may be convenient to use the Method Wizard to build methods for different applications.

The Method Wizard can be used in two different ways:

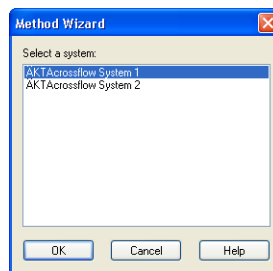
- When a method is created in the **Method Editor** of **UNICORN**. The Method Wizard dialogs are passed and finally the method is saved.
- When an **Instant Run** is performed. In this case the Method Wizard dialogs are passed before the run is performed. The method is not saved in this procedure. See Section 9.3.

To create a new method using the Method Wizard in the Method Editor:

Note: Depending on used filter type in the method (Hollow fiber or Flat Sheet), remember to select correct component according to instruction in Section 2.4.



- 1 In the **Method Editor** of **UNICORN**, click the Method Wizard icon.
- 2 Select System.

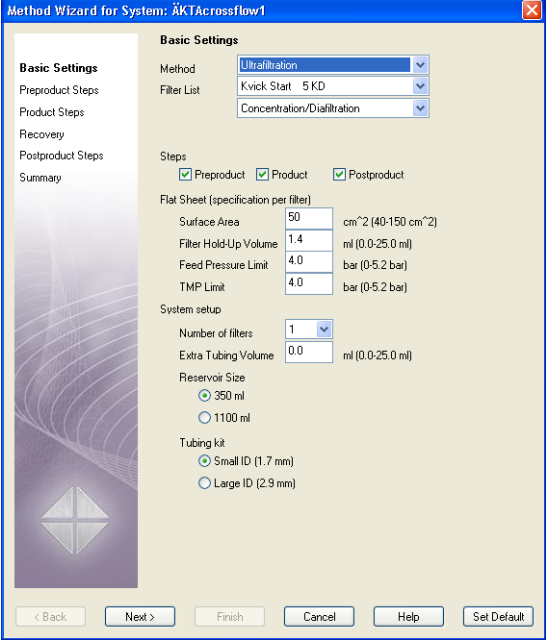


3 A dialog of **Basic Settings** will first be displayed.

Note: *This example is from when "Flat Sheet" is selected as component.*

- To obtain default values in the Method Wizard, click **Set Default**. This is only possible in the first dialog.
- In the **Method** list, the type of process is selected.
- The **Filter List** section will display available GE Healthcare filters. The used filter type is selected here.
- Below the **Filter List**, the type of method and included steps are selected.
- The **Flat Sheet (specification per filter)** area displays the recommended default values for the selected filter type. These can be edited by the user.
- In the **System setup** section, it is possible to select number of filters and extra tubing volume. This is necessary when using several filters assembled together in parallel. The "Extra tubing volume" has to be calculated manually if other tubing than standard are used.
- Reservoir size and tubing kit to be used in the recycling loop is selected.
- To get help instructions for each Method Wizard dialog, click **Help**, or press the **F1** key.
- To go back to the previous dialog, click **Back**.
- To stop the Method Wizard, click **Cancel**.

- To proceed with the next dialog, click **Next**.



Method Wizard for System: ÄKTAcrossflow1

Basic Settings

Method: Ultrafiltration
Filter List: Kwick Start 5 KD
Concentration/Diafiltration

Steps
☒ Preproduct ☒ Product ☒ Postproduct

Flat Sheet (specification per filter)
Surface Area: 50 cm² (40-150 cm²)
Filter Hold-Up Volume: 1.4 ml (0.0-25.0 ml)
Feed Pressure Limit: 4.0 bar (0-5.2 bar)
TMP Limit: 4.0 bar (0-5.2 bar)

System setup
Number of filters: 1
Extra Tubing Volume: 0.0 ml (0.0-25.0 ml)
Reservoir Size
☒ 350 ml
☐ 1100 ml
Tubing kit
☒ Small ID (1.7 mm)
☐ Large ID (2.9 mm)

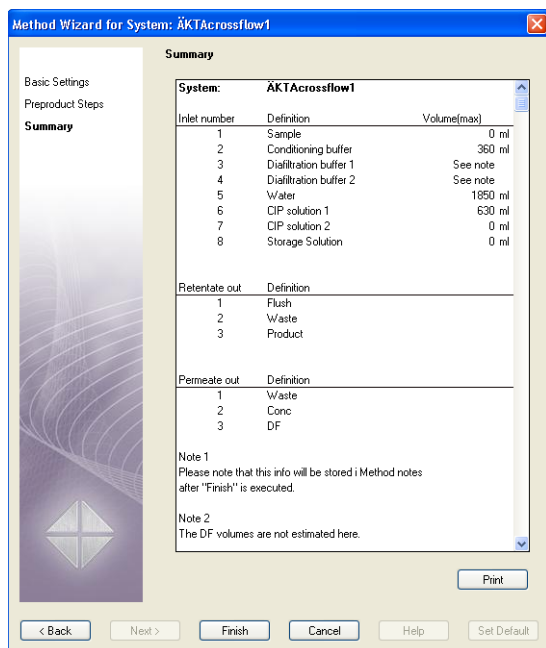
< Back Next > Finish Cancel Help Set Default

- 4 In each dialog, select the appropriate parameter values and click **Next** to continue.

- 5 After a number of dialogs, depending on selections, a **Summary** dialog is shown.

- A list of calculated volumes of required solutions is displayed.

Note: To prepare a run with solutions, we recommend printing this list using the **Print** button. This list will also be displayed in **Method Notes** in the **Run Setup** dialog.



Method Wizard for System: ÄKTAcrossflow1

Basic Settings
Preproduct Steps
Summary

Summary

Inlet number	Definition	Volume(max)
1	Sample	0 ml
2	Conditioning buffer	360 ml
3	Dialfiltration buffer 1	See note
4	Dialfiltration buffer 2	See note
5	Water	1850 ml
6	CIP solution 1	630 ml
7	CIP solution 2	0 ml
8	Storage Solution	0 ml

Retentate out

Definition
1 Flush
2 Waste
3 Product

Permeate out

Definition
1 Waste
2 Conc
3 DF

Note 1
Please note that this info will be stored i Method notes after "Finish" is executed.

Note 2
The DF volumes are not estimated here.

Print

< Back Next > Finish Cancel Help Set Default

- 6 Click **Finish** in the **Summary** dialog.

7 The **Run Setup** window that appears consists of a number of tab pages. On the variable page, chosen running conditions are displayed.

- To show detail variables, check **Show details** box. Detail variables are indicated by a **D** in the column immediately to the left of the **Variable** column.
- To show unused variables, check **Show unused variables** box. Unused variables are indicated by a **U** in the column immediately to the left of the **Variable** column.
- To edit a variable's value, click in the cell and change the value.

Note: If a variable's value is changed, it will affect the created method. A changed variable may also affect the calculated required solution volumes in the previous **Summary** dialog.

Note: We do recommend to not edit any variables, especially not the detail variables.

Note: Do not delete variables, with the **Edit Variable:Delete** command.

Block	Variable	Value	Range
Start_Settings_System	TMP_Alarm (bar)	4.00	0.00 - 5.20
	Retentate_Holdup (ml)	19.00	-1000.00 - 1000.00
Alarm_FeedPress	Feed_Press_Alarm (bar)	4.00	0.00 - 5.20
Empty_Reservoir_Completely	FeedPress_EmptyRes (bar)	3.00	0.00 - 5.20
	MaxFlow_EmptyRes (ml/min)	600.0	0.0 - 600.0
Start_Settings_FS	Filter_Area (cm²)	50.0	1.0 - 1200.0
Rinse_RVB_Vol	Rinse_Ret_Vol (ml)	50.00	0.00 - 999999.00
Rinse_Vol	Rinse_Volume (ml)	300.00	0.00 - 999999.00
CIP_Flux	CIP_Max_Flux (LMH)	30.0	0.0 - 4800.0
	TMP_Limit_CIP (bar)	4.00	0.01 - 5.20
Time_PreCIP1	Pre_CIP1_Time (min)	60.00	0.00 - 999999.00
Flush_Vol	Flush_Volume (ml)	300.00	0.00 - 999999.00
TMP_PreWaterFluxTest	TMP_PreWaterFlux (bar)	1.00	0.00 - 5.20
BufferCond_Fill_Vol	BufferCond_Fill_Vol (ml)	130.00	0.00 - 999999.00

☐ Show details
☐ Show unused variables
☒ Display tooltip for extended variable cells

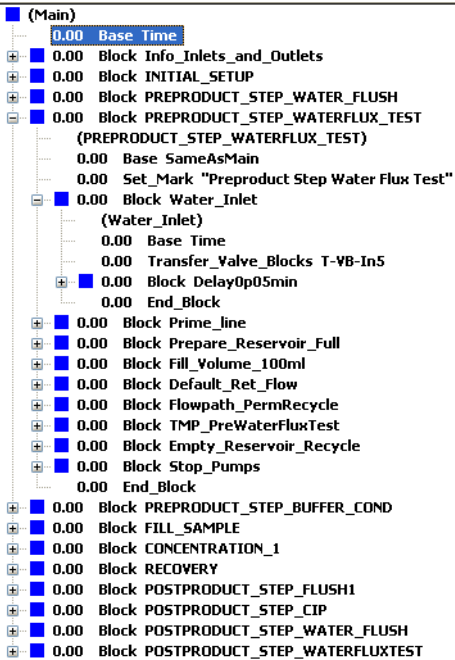
Edit Variable... Help

8 In the **Run Setup** dialog, there are tabs to select to for example make notes, enter method information and make selections of start protocol.



9 To display the text instructions of the created method, click the Text Instruction icon.

- 10 To view and expand the method, click the “plus”. An alternative is to use the keyboard arrow keys.

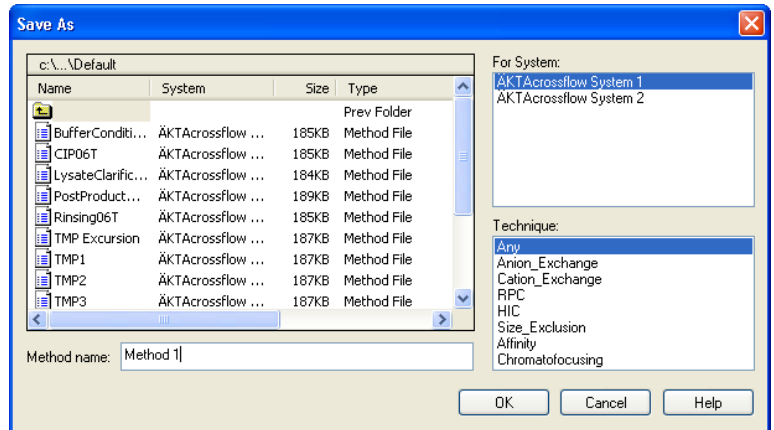


- 11 The method can be edited in the Text editor as described in Section 3.3.
- 12 To save the method, select **File:Save**.

13 Select location.

14 Enter method name, select System and Technique. Click **OK**.

Note: "Any" must be selected as Technique.



4 Performing cross flow runs manually

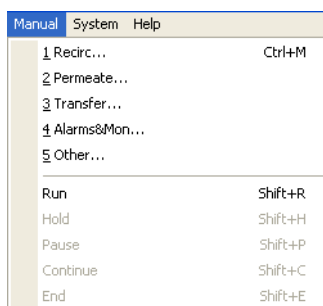
4.1 Introduction

The most convenient way to perform cross flow runs is of course to use Method Wizard created methods. However, it is always possible to perform process steps using the manual mode in UNICORN.

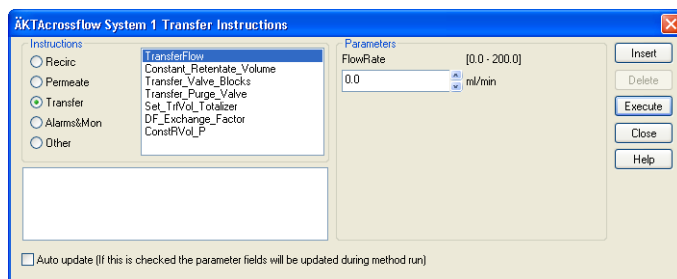
Note: All Strategy text instructions and parameters are listed in Chapter 13.

4.2 Executing text instructions

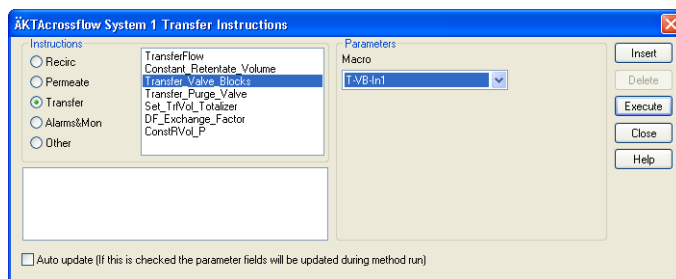
- 1 In the **System Control** window in UNICORN, select the desired instruction group from the menubar under **Manual**.



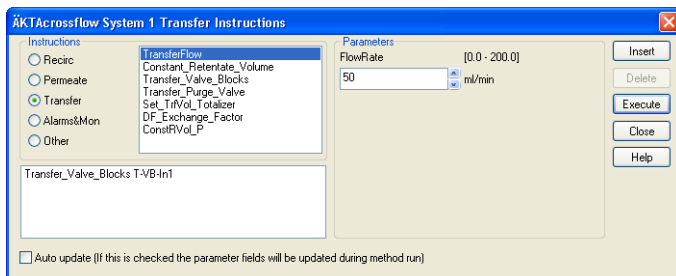
An instruction box is displayed.



- 2 Select instruction group, for example Transfer.
- 3 Select parameter, for example Transfer_Valve_Blocks.
- 4 Select an inlet valve.
- 5 Click **Insert**.



- 6 Select parameter, for example Transfer_Flow.
- 7 Enter a parameter value, for example 50 ml/min.



- 8 Click **Insert**.
- 9 When all commands are set for the desired operation, click **Execute**.
- 10 To stop the operation, click the **End** or **Pause** button in the **System Control** window.

Note: Reset *ResVol_Totalizer* under *Recir* to avoid values below zero.

4.2.1 WARNINGS and CAUTIONS

CIP method

When running a method using a CIP (Clean-in-place) solution containing sodium hydroxide (NaOH):



WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective lab coat.

CAUTION! Always make sure that the filters and system components are compatible with sodium hydroxide at the concentration, time and temperature used.

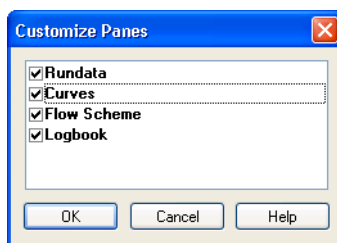
General



WARNING! Do not operate the ÄKTAcrossflow system at pressures above the specified maximum pressure (5.2 bar).

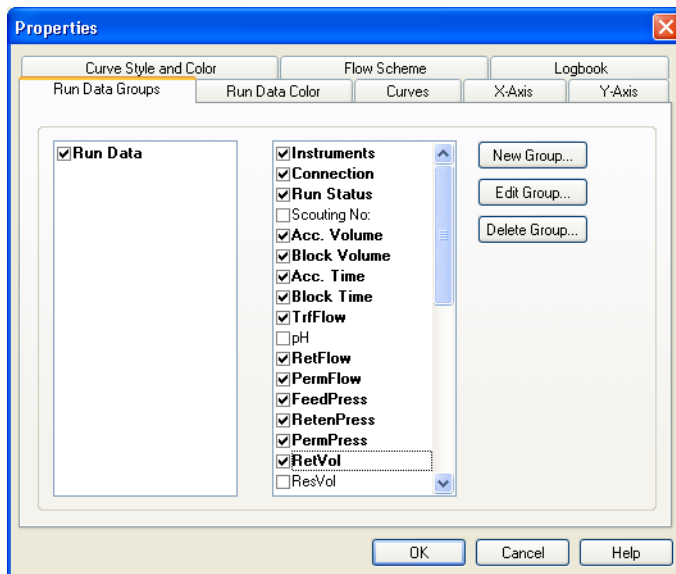
4.3 Monitoring the run

- 1 In **System Control** in UNICORN select **View:Panels**.
- 2 Check the desired boxes and click **OK**.

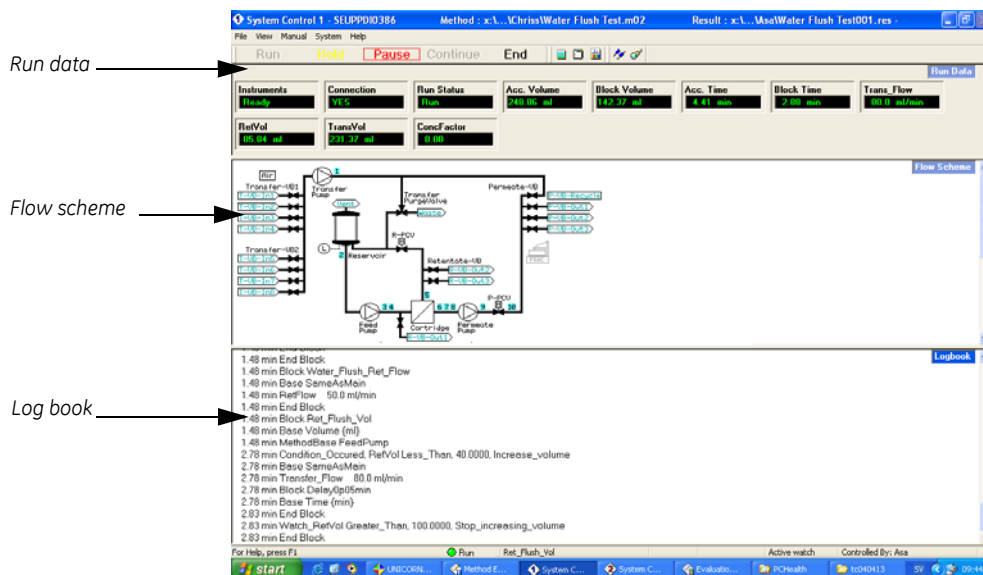


- 3 To edit the displayed panes, right-mouse-click in the window and select **Properties**.

- 4 Select appropriate tab and choose how to display data.



Example of displayed information:



5 Creating Preproduct methods using the Method Wizard

5.1 Preproduct methods: Introduction

There are different methods which are used before the actual cross flow run is performed:

- Rinsing
- Filter CIP (Clean-in-place)
- Water Flush
- Water Flux test, and
- Buffer conditioning

Preproduct Method	Description	Method Wizard dia-logs	Visualized Procedure
Basic Settings: Flat Sheets		Section 5.3.1, p.73	
Basic Settings: Hollow Fibers		Section 5.3.2, p.75	
Rinsing	Section 5.2.1, p.69	Section 5.3.3, p.77	Section 5.4.3, p.84
Filter CIP	Section 5.2.2, p.69	Section 5.3.3, p.78	Section 5.4.4, p.84
Water Flush	Section 5.2.3, p.71	Section 5.3.3, p.78	Section 5.4.5, p.86
Water Flux test	Section 5.2.4, p.71	Section 5.3.3, p.78	Section 5.4.6, p.88
Buffer conditioning	Section 5.2.5, p.72	Section 5.3.3, p.79	Section 5.4.7, p.89
"Preparing system and reservoir" procedure	Dependent on previous method, a procedure which some of the methods begin with.		Section 5.4.1, p.80
"Preparing system" procedure	Dependent on previous method, a faster procedure which some of the methods begin with.		Section 5.4.2, p.82

Table 5-1. Table of Cross-references.

Depending on how the system and/or filter was treated the day before, different combinations of the methods above may be used.

If the system has not been cleaned for a while, it may be necessary to start with a System sanitization. See Section 10.1. To avoid contaminations when a different protein is introduced, it may also be necessary to perform a System sanitization.

Condition	Rinsing	Filter CIP 1	Water Flush	Filter CIP 2	Water Flush	Water Flux Test	Buffer Conditioning
New filter	X					X	X
After "Recommended postproduct procedure" and same filter							X
After "Recommended postproduct procedure" but other filter					X	X	X

Table 5-2. Preproduct steps

Condition	Flush (Water/ Buffer)	Filter CIP 1	Water Flush	Filter CIP 2	Water Flush	Water Flux Test	Filter Storage Solution
Recommended postproduct procedure	X	X	X	X	X	X	X

Table 5-3. Postproduct steps.

5.2 Preproduct methods: Description

5.2.1 Rinsing

A new filter for ultrafiltration is stored in NaOH and Glycerol, which means it has to be rinsed with an increased volume to get rid of the glycerol. Use the Rinsing method. However, a new filter does not need to be washed with a CIP method. The method starts with "Preparing the system" block.

The method will rinse:

- The water inlet tubing in the transfer section.
- Retentate loop.
- Reservoir.
- Retentate and permeate side of filter.

Hollow fibers for microfiltration are dry at delivery. However, they need to be wetted with the Rinsing method before use.

Note: *Some Hollow fibers might not wet out very well with water. If problems occur, for example a failed water flux test, it can be necessary to flush the filter manually with 10% Isopropyl alcohol. For more information, refer to the Hollow fiber operating guide.*

5.2.2 Filter CIP

A filter which has been used in a process of a protein, should be cleaned with a Filter CIP method.

The Filter CIP method includes an option to perform two Filter CIP procedures with an optional water flush between.

The created method will:

- Wash the transfer line.
- Wash the reservoir.

Note: *If the Filter CIP method is a postproduct step, the system and the reservoir will be cleaned ("Preparing system and reservoir"), see Section 5.4.1. If the method is a preproduct step, the system will be cleaned ("Preparing system"), see Section 5.4.2.*

- Wash the filter, both on retentate and permeate side with CIP solution.
 - If large reservoir is used, Volume to fill has to be entered.
-

-
- Circulate at set time.
 - Empty the system of CIP solution.

Note: *The system will not be sanitized. For sanitization of the system, see Section 10.1.*

5.2.3 Water flush

A used filter can be rinsed with the Water flush method. It is also the recommended method to be performed after a Filter CIP method where NaOH was used. The method should also be used if a filter has not been rinsed with water before a water flux test is performed.

The method will rinse:

- The water inlet tubing in the transfer section.
- Retentate loop.
- Reservoir.

Note: *If the previous method is Filter CIP, or the Water flush method is a post product step, system and reservoir will be washed. Otherwise, only system will be washed.*

- Retentate and permeate side of filter.

5.2.4 Water Flux test

Note: *Water flush or Rinsing has to be performed before Water Flux test.*

This is a method to have control of the quality status of the membrane cartridge. By comparing obtained water fluxes as a function of time of usage, it is possible to see when it is necessary to extensively clean or discard the filter.

We recommend performing the test:

- before a product step, and
- after a product step and filter cleaning.

The way to perform the test by used control mode is dependent on the filter type used. Default for flat sheets is:

- TMP control mode.
- A TMP value for the water flux test is entered.

Default for hollow fibers with cut off smaller than 0.1 µm is:

- TMP control mode.

Default for hollow fibers with cut off 0.1 µm and larger is:

- NFF (Normal Flow Filtration) control
- Feed flow or Feed pressure is selected as Feed control and a value is entered.

Note: *When comparing status of a filter as a function of time and number of experiments, it is important to have performed the same type of method, i.e. same TMP/NFF feed control value.*

The principle of the test method is:

- 1 If control mode is TMP, DeltaP is set. NFF control, starts the feed pump it self.
- 2 When the TMP/NFF has been reached, and the flux is stabilized for 3 minutes a normalized water flux is measured and an instruction Set_Eval_Mark with the parameter Normalized_Water_Flux is set.

The data is analyzed by the Evaluation module of UNICORN. For example, normalized water flux is plotted against number of performed runs with the filter. Temperature effect due to viscosity is compensated for using a correction table. Results are compared to previous tests and provide information about the quality status of the filter.

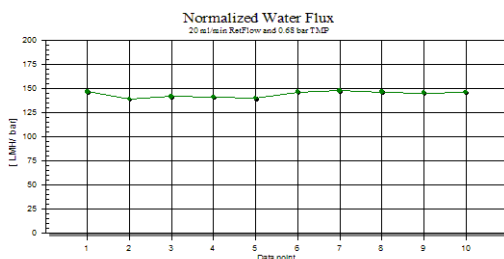


Fig 5-1. Example of plotted Normalized Water Flux.

Note: Some Hollow fibers might not wet out very well with water. If problems occur, for example a failed water flux test, it can be necessary to flush the filter manually with 10% Isopropyl alcohol. For more information, refer to the Hollow fiber operating guide.

5.2.5 Buffer conditioning

The purpose of the method is to replace the water in the system with a buffer which is suitable for the protein/cell in the planned Product filtration run. The created method will:

- Fill the system with the selected buffer.
- Condition the filter with buffer.
- The first block in the method is "Preparing the system".

Note: If a CIP has been performed, we recommend performing a buffer conditioning after the water flush to ensure that the pH in the system is OK before starting a product method.

5.3 Preproduct methods: Method Wizard dialogs

Note: To set default values, click the **Set Default** button in the first dialog. To get help instructions for a dialog, click **Help** or press **F1**.

5.3.1 Basic settings: Flat sheets

Note: To get the Method Wizard for flat sheets, "Flat-sheet" has to be selected as component in System Setup. See Section 2.4.

Method Wizard for System: ÅKTAcrossflow1

Basic Settings

Method: Ultrafiltration

Filter List: Kwick Start 5 KD

Concentration/Diafiltration

Steps:

☒ Preproduct ☒ Product ☒ Postproduct

Flat Sheet (specification per filter)

Surface Area: 50 cm² (40-150 cm²)

Filter Hold-Up Volume: 1.4 ml (0.0-25.0 ml)

Feed Pressure Limit: 4.0 bar (0-5.2 bar)

TMP Limit: 4.0 bar (0-5.2 bar)

System setup

Number of filters: 1

Extra Tubing Volume: 0.0 ml (0.0-25.0 ml)

Reservoir Size:

☒ 350 ml

☐ 1100 ml

Tubing kit:

☒ Small ID (1.7 mm)

☐ Large ID (2.9 mm)

< Back Next > Finish Cancel Help Set Default

- In the **Method** list, select Ultrafiltration.

Basic Settings

Method: Ultrafiltration

Filter List:

Calibrate level sensor

Installation Test

System Sanitization

Ultrafiltration

- In the **Filter List**, available GE Healthcare filters are displayed. Select the used filter or select Other.

Method: Ultrafiltration

Filter List:

Kwick Start 5 KD

Kwick Start 10 KD

Kwick Start 10 KD Select

Kwick Start 30 KD

Kwick Start 50 KD

Kwick Start 100 KD

Other...

Steps:

☒ Preproduct

- In the next list, select Concentration/Diafiltration.

Basic Settings

Method	Ultrafiltration
Filter List	Kwick Start 5 KD - 50 cm ²
	Concentration/Diafiltration
	Concentration/Diafiltration
	UF Process Optimization

- In the next area, select the type of process step.

Steps

☒ Preproduct ☐ Product ☐ Postproduct

- The **Flat Sheet (specification per filter)** area displays the recommended default values for the selected filter type. Accept or edit the values.

Flat Sheet (specification per filter)

Surface Area	50	cm ² (40-150 cm ²)
Filter Hold-Up Volume	1.4	ml (0.0-25.0 ml)
Feed Pressure Limit	4.0	bar (0-5.2 bar)
TMP Limit	4.0	bar (0-5.2 bar)

If “Other” filter type has been selected, enter Surface Area, Hold Up Volume, Feed pressure and TMP limits for the flat sheet. Information is usually available from the manufacturer.

Note: When using TMP in a method, a value close to “TMP limit” may lead to the run being paused. Always choose a lower working TMP than the “TMP limit”.

- If several filters are assembled together in parallel, select in the **System setup** section the Number of filters (1-3). If only one filter is used, keep the default value “1”.
- If other tubing than standard is used, calculate the extra tubing volume and enter the value in the “Extra Tubing Volume” box.

System setup

Number of filters	1
Extra Tubing Volume	0.0 ml (0.0-25.0 ml)

- Select Reservoir size and Tubing kit used in the recycle loop.

Reservoir Size

☒ 350 ml

☐ 1100 ml

Tubing kit

☒ Small ID (1.7 mm)

☐ Large ID (2.9 mm)

5.3.2 Basic settings: Hollow fibers

Note: To get the Method Wizard for hollow fibers, "Hollow fiber" has to be selected as component in System Setup. See Section 2.4.

- In the **Method** list, select Ultrafiltration/Cell Processing.

- Select Filter Type, AXM or AXH. AXM hollow fibers have a straight linear shape. AXH hollow fibers are curved into a loop.

- In the **Filter List**, available GE Healthcare filters are displayed. Select the used filter or select Other.

Basic Settings

Method: Ultrafiltration/Cell Processing

Filter Type: AXM

Filter List: AXM 3 KD - 0.50 mm

- AXM 3 KD - 0.50 mm
- AXM 10 KD - 0.50 mm
- AXM 30 KD - 0.50 mm
- AXM 100 KD - 0.50 mm
- AXM 300 KD - 0.50 mm
- AXM 500 KD - 0.50 mm
- AXM 500 KD - 1.00 mm
- AXM 750 KD - 1.00 mm
- AXM 0.10 µm - 1.00 mm
- AXM 0.20 µm - 1.00 mm
- AXM 0.45 µm - 1.00 mm
- AXM 0.65 µm - 0.75 mm
- Other...

- Select the type of process step.

Steps

☒ Preproduct ☐ Product ☐ Postproduct

- The **Hollow Fibre (specification per filter)** area displays the recommended default values for the selected filter. Accept or edit the values.

Hollow Fibre (specification per filter)

Lumen Diameter	0.5	mm (0.10-10.00 mm)
Number of Fibers	12	(1-1000)
Surface Area	50	cm ² (40-150 cm ²)
Lumen Hold-Up Vol	1.17	ml (0.0-25.0 ml)
Feed Pressure Limit	4.3	bar (0-5.2 bar)
TMP Limit	3.0	bar (0-5.2 bar)

If “Other” filter has been selected, enter Lumen diameter, Number of fibers, Surface area, Lumen hold-up volume, Feed pressure limit and TMP limit for the hollow fiber. Information is usually available from the manufacturer.

Note: When using TMP in a method, a value close to “TMP limit” may lead to the run being paused. Always choose a lower working TMP than the “TMP limit”.

- If several filters are assembled together in parallel, select in the **System setup** section the Number of filters (1-3). If one filter is used only, keep the default value “1”.
- If other tubing than standard is used, calculate the extra tubing volume and enter the value in the “Extra Tubing Volume” box.

System setup

Number of filters: 1

Extra Tubing Volume: 0.0 ml (0.0-25.0 ml)

- Select Reservoir size and Tubing kit used in the recycle loop.

Reservoir Size

☒ 350 ml

☐ 1100 ml

Tubing kit

☒ Small ID (1.7 mm)

☐ Large ID (2.9 mm)

5.3.3 Preproduct setup

Method Wizard for System: ÄKTAcrossflow2

Basic Settings

Preproduct Steps

Summary

Preproduct setup

☒ Rinsing

☒ Filter CIP

☒ CIP 1

Length of Time min (0.9999 min)

☒ CIP 2

Length of Time min (0.9999 min)

☒ Water Flush between CIP

☒ Water Flush

☒ Water Flux Test

☒ TMP

☐ NFF

bar (0.5-2 bar)

☒ Buffer Conditioning

< Back Next > Finish Cancel Help Set Default

Rinsing:

- To include a Rinsing in the method, check the **Rinsing** box.

☒ Rinsing

Filter CIP

- To include a Filter CIP, check the **Filter CIP** box.
- There is an option to select two CIP with a optional Water flush between.
- The circulation time of CIP solution is entered in the **Length of Time** boxes.

☒ Filter CIP
☒ CIP 1
 Length of Time min (0-9999 min)
☒ CIP 2
 Length of Time min (0-9999 min)
☒ Water Flush between CIP

- If the large reservoir is used, "Volume to Fill" has to be entered.

Note: The minimum volume is 200 ml.

Volume to Fill ml (200-1100 ml)
 Length of Time min (0-9999 min)
☐ CIP 2

Water flush

- To include a Water flush in the method, check the **Water Flush** box.

Note: After a Filter CIP, we recommend running a water flush.

Note: If a Water Flux test will be performed, Rinsing or Water Flush should be performed before the test.

☒ Water Flush

Water Flux test

- To include a Water Flux test in the method, check the **Water Flux Test** box.
- Select TMP or NFF (Normal Flow Filtration) as control mode. NFF is default for hollow fibers with cut off 0.1 μm and larger.
- For TMP as control mode:
 - Enter a TMP value for the test.

Note: When comparing status of a filter as a function of time and number of experiments, it is important to have performed the same type of method, (i.e. same TMP value).

☒ Water Flush
☒ Water Flux Test
☒ TMP bar (0-5.2 bar)
☐ NFF

- For NFF as control mode:
 - Select to perform Feed control for NFF with FeedFlow or FeedPressure.

The screenshot shows the 'Feed Control for NFF' section of the Method Wizard. It includes checkboxes for 'Water Flush' and 'Water Flux Test', both of which are checked. Below these are radio buttons for 'TMP' and 'NFF', with 'NFF' selected. To the right, a dropdown menu is open, showing 'FeedPressure' as the selected option, with 'FeedFlow' and 'FeedPressure' as visible choices. The text 'Feed Control for NFF' and the range '00-5.20 bar' are also visible.

- Enter a value for the selected Feed control.

The screenshot shows the 'Feed Control for NFF' section of the Method Wizard. It includes checkboxes for 'Water Flush' and 'Water Flux Test', both of which are checked. Below these are radio buttons for 'TMP' and 'NFF', with 'NFF' selected. To the right, a dropdown menu is open, showing 'FeedFlow' as the selected option, with 'FeedFlow' and 'FeedPressure' as visible choices. The text 'Feed Control for NFF' is visible. Below the dropdown, a text box contains the value '0.69' and the unit 'ml/min (0-200 ml/min)'.

Buffer conditioning

- To include a Buffer conditioning in the method, check the **Buffer Conditioning** box.

Note: We recommend performing a Buffer conditioning before a Product filtration run, especially when a Filter CIP has previously been performed.

The screenshot shows the 'Buffer Conditioning' checkbox, which is checked. The text 'Buffer Conditioning' is visible next to the checked box.

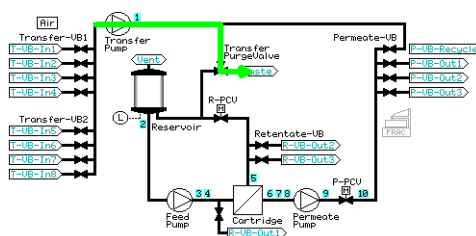
5.4 Preproduct methods: Visualized procedures

Note: For information on the specific instructions, for example Constant Retentate Volume, see Chapter 13.

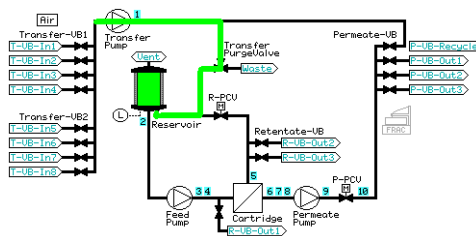
5.4.1 Visualized procedure of Preparing the system and the reservoir

Depending on the previous step, some of the methods begin with a preparation of the system and reservoir. However, different transfer inlet tubing is used depending on the preferred liquid.

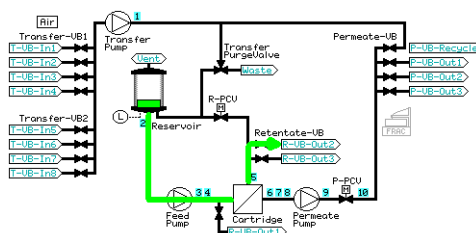
- 1 Transfer pump fills the tubing from Transfer Inlet to Transfer Purge Valve-Waste.



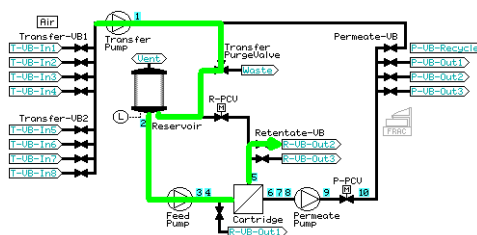
- 2 Reservoir is completely filled to 350 ml.



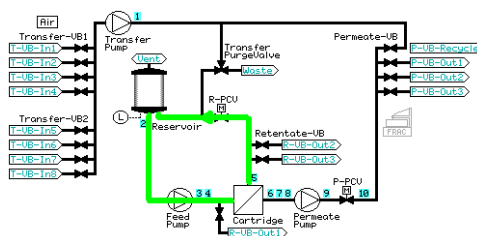
- 3 The reservoir is emptied through R-VB-Out2 (Waste).



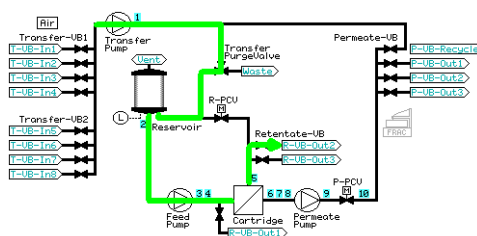
- 4 At Constant Retentate Volume (reservoir volume 5 ml), transfer flow = feed flow, 50 ml is pumped at the retentate side out to R-VB-Out2.



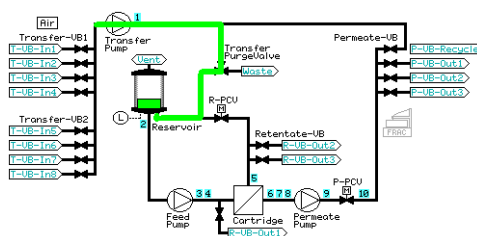
- 5 To rinse the distance between Retentate valve block and reservoir, the Retentate VB is set to recirculation, 25 ml/min 15 ml.



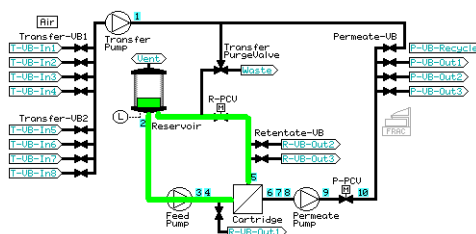
- 6 5 ml is emptied and the reservoir is filled with 5 ml.
- 7 At Constant Retentate Volume, a new flush is performed out to R-VB-Out2.



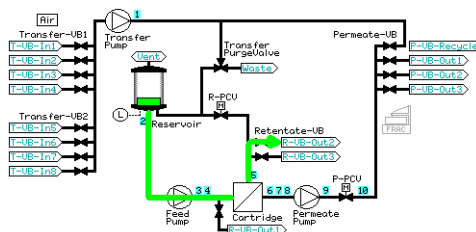
- 8 The reservoir is filled with a volume, defined by the used filter area.



- 9 Recirculation at the retentate side for one minute with FeedPressure control.



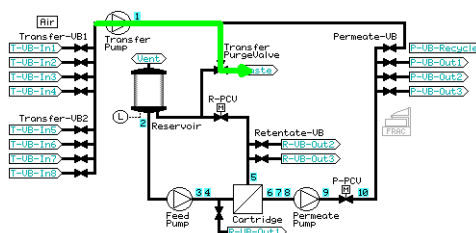
- 10 Then, the reservoir is emptied through R-VB-Out2.



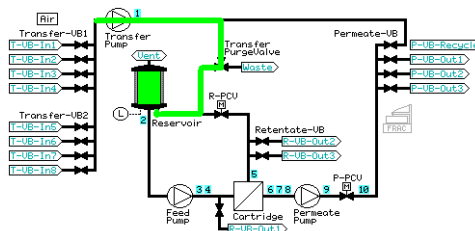
5.4.2 Visualized procedure of Preparing the system

Depending on previous step, some of methods begin with a "Preparing the system". This method is faster than "Preparing the system and reservoir", and is used when the reservoir does not need to be treated. However, different transfer inlet tubing is used dependent of preferred liquid.

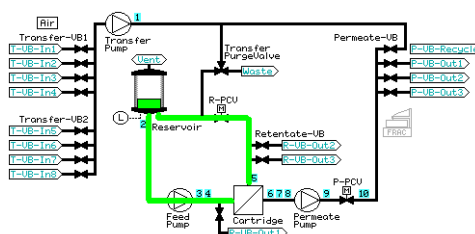
- 1 Transfer pump fills the tubing from Transfer Inlet to Transfer Purge Valve-Waste.



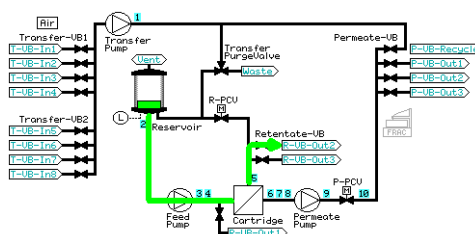
- 2 Reservoir is filled to a volume, defined by used filter area.



- 3 25 ml of the reservoir volume is emptied.
- 4 Recirculation at the retentate side for one minute with FeedPressure control.

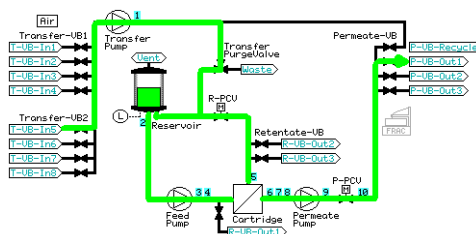


- 5 The reservoir is emptied through R-VB-Out2 (Waste).

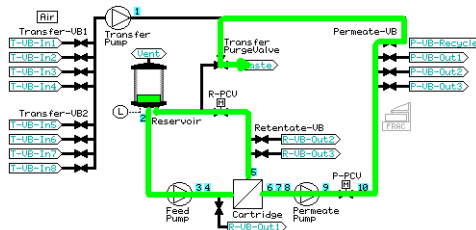


5.4.3 Visualized procedure of the rinsing method

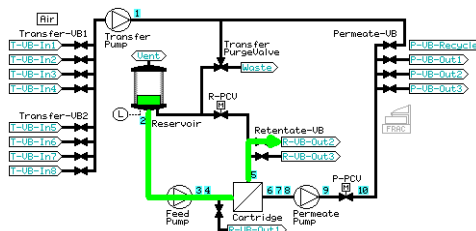
- 1 The system is prepared according to the procedure "Preparing the system" described in Section 5.4.1. The Transfer Inlet used is T-VB-In5 (water).
- 2 The reservoir is filled with 100 ml water. At Constant Retentate Volume and TMP regulation, a volume, defined by filter type and area, is passed through the filter out to P-VB-Out1 (Waste).



- 3 Constant Retentate Volume is disabled. 50 ml of the reservoir volume is emptied through the filter. P-VB is set to recirculation and the liquid leaves the system through Transfer Purge Valve-Waste.



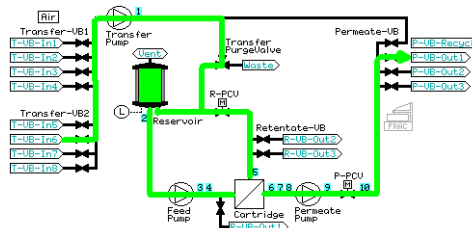
- 4 The rest of the reservoir volume is emptied through R-VB-Out2. At level 0 ml in the reservoir, the rinsing is complete.



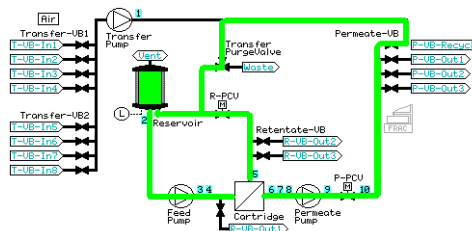
5.4.4 Visualized procedure of the Filter CIP method

- 1 If the method is a post-product method, the system and reservoir are prepared according to the procedure "Preparing the system and reservoir", described in Section 5.4.1. The Transfer Inlet used is T-VB-In6 (CIP solution). If the method is a pre-product method, "Preparing the system" will be used.

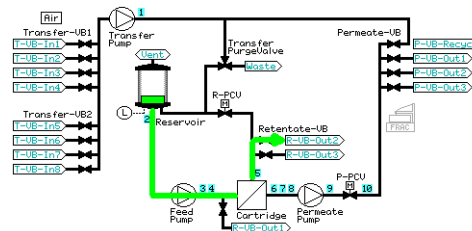
- _____



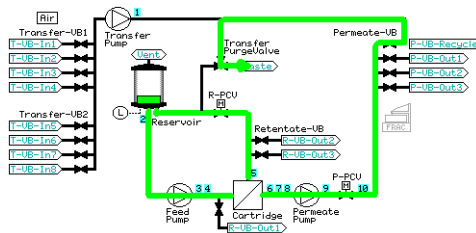
- After 30 ml, the permeate valve is set to P-VB-Recycle and the liquid is recycled to the reservoir.



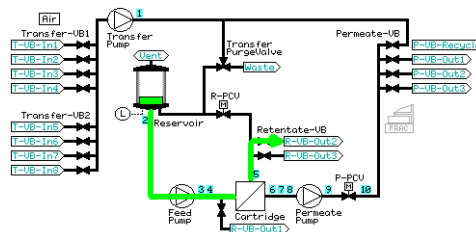
- 4 After the specified CIP 1 circulation time, for example 30 min, ConstRetVol is disabled and the reservoir is emptied through R-VB-Out2.



- 5 If Water Flush between CIP has been selected, the reservoir is filled with 100 ml water and 50 ml leaves via Permeate recycle to Transfer Purge Valve-Waste.



- 6 The remaining 50 ml leaves the system through R-VB-Out2.



- 7 Then if a CIP 2 has been selected, the reservoir is filled with 100 ml CIP2 solution and the procedure described above is repeated, except for that less volumes are used.

5.4.5 Visualized procedure of the Water flush method

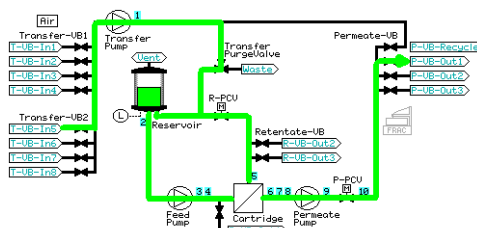
If the previous method was Filter CIP or if the method is a post product method, the system and reservoir are prepared according to the procedure "Preparing the system and reservoir", described in Section 5.4.1.

Otherwise the system will be prepared according to the procedure "Preparing the system".

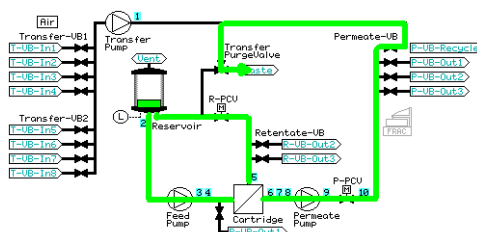
- 1 The reservoir is prepared according to the procedure described in Section 5.4.1. The Transfer Inlet used is T-VB-In5 (water).

- 2 The reservoir is filled with 100 ml water. At Permeate_Unrestricted_Flow, FeedPressure Control and Constant_Retentate_Volume, a volume, defined by used filter, is passed through the filter out to P-VB-Out1.

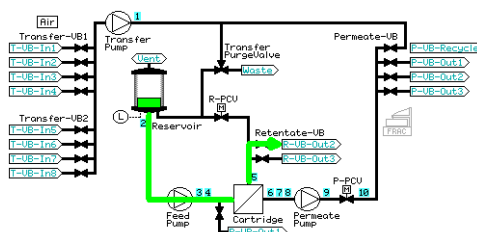
For hollow fibers, 750 kD and smaller, TMP control is used. For 0.1 µm and larger, Flux control is used.



- 3 “Permeate Unrestricted Flow” is disabled. 50 ml is emptied through the filter. P-VB is set to recirculation and the liquid leaves the system through Transfer Purge Valve-Waste.



- 4 The rest of the reservoir liquid is emptied through R-VB-Out2. At level 0 ml in the reservoir, the water flush is complete.

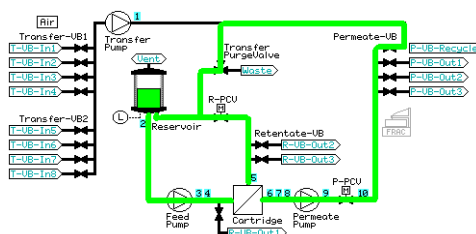


5.4.6 Visualized procedure of the Water flux test

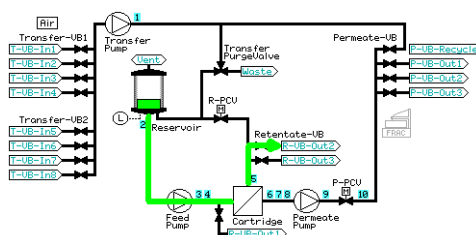
The filling of reservoir procedure is included in the previous method, which should be "Rinsing" or "Water flush". So, the Water flux test is just a continuation of these methods. If the Waterflux test is used as a single method, "Prepare system" will be the first step. Additional 50 ml will be used for the test.

- 1 The permeate valve block is set to recirculation and the transfer purge valve is set to reservoir. A permeate flow is started by TMP control. When a stable flux has been achieved, a value of a "normalized water flux" is collected by setting a Set_Eval_Mark with the parameter Normalized_Water_Flux.

If Normal flow filtration (NFF) is used as control mode, Feed flow is not necessary.

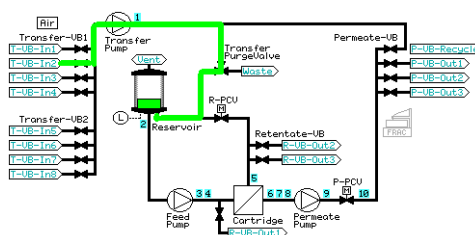


- 2 If Water flux test is a part of Water flush or Rinse, the method will be ended as these methods. If the test is performed as a single method, the reservoir will be emptied using the function EmptyReservoir.
- 3 The rest of the reservoir liquid is emptied through R-VB-Out2. At level 0 ml in the reservoir, the test is complete.

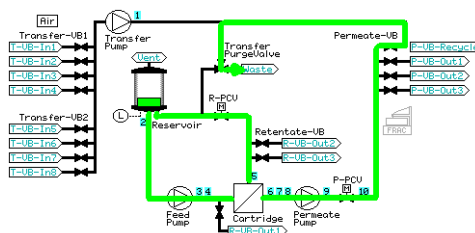


5.4.7 Visualized procedure of the Buffer conditioning method

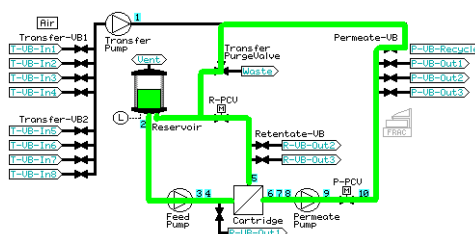
- 1 The system is prepared according to the procedure "Preparing the system" described in Section 5.4.1. The Transfer Inlet used (T-VB-In2) is connected to buffer.
- 2 The reservoir is filled with a volume, defined by used filter area.



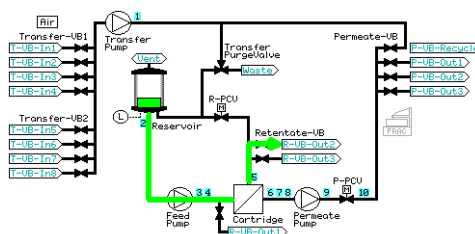
- 3 Permeate recycle to waste is opened. The permeate pump is started using PUF control (Flat sheets) or TMP/Flux control (Hollow fibers). 30 ml is emptied through Transfer Purge Valve-Waste. Then, the valve is set to reservoir.



- 4 Recirculation for 5 minutes.



- 5 The rest of the reservoir liquid is emptied through R-VB-Out2.





6 Process optimization in Ultrafiltration

6.1 Introduction

A TMP excursion method can be created to determine the optimal cross flow and TMP settings for an ultrafiltration process of a specific protein.

6.2 Experiment plan

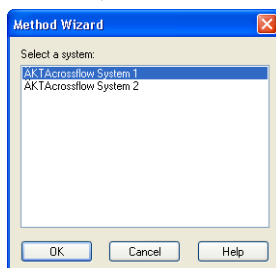
The goal of an optimization can for example be to find the optimal conditions for a 10x concentration of a 5 mg/ml antibody protein solution to 50 mg/ml. The experiment plan includes therefore different Retentate flows (Q_R) and TMP settings for the two antibody concentrations.

Antibody concentration (mg/ml)		Set1	Set2	Set3
5	Q_R (ml/min)	24	16	8
	TMPsetpoints (bar)	1.0-1.6-2.2-2.8-3.4		
50	Q_R (ml/min)	24	16	8
	TMPsetpoints (bar)	1.0-1.6-2.2-2.8-3.4		

6.3 Creating a method



- 1 Open the Method Wizard by clicking the Wizard icon in the **Method Editor** in UNICORN.
- 2 Select a system and click **OK**.



3 In the **Basic Settings** dialog:

- Select Ultrafiltration as method
- Select the used filter from the list of available GE Healthcare filters, or select "Other".
- Select UF Process Optimization
- In the Flat Sheet (specification per filter) section, recommended default values are displayed. Accept these values or edit them.
- If "Other" has been selected as filter, enter parameters for the used flat sheet cassette. Check information from filter manufacturer.
- If several filters, assembled together in parallel, are used, enter number of filters. If other tubing than standard is used, calculate the extra volume and enter the value.
- Select reservoir size and tubing kit used in the recycle loop.

Basic Settings

Method: Ultrafiltration

Filter List: Kwick Start 5 KD

UF Process Optimization

Flat Sheet (specification per filter)

Surface Area	50	cm ² (40-150 cm ²)
Filter Hold-Up Volume	1.4	ml (0.0-25.0 ml)
Feed Pressure Limit	4.0	bar (0-5.2 bar)
TMP Limit	4.0	bar (0-5.2 bar)

System setup

Number of filters: 1

Extra Tubing Volume: 0.0 ml (0.0-25.0 ml)

Reservoir Size

☒ 350 ml

☐ 1100 ml

Tubing kit

☒ Small ID (1.7 mm)

☐ Large ID (2.9 mm)

4 Click **Next**.

5 In the **UF Process Optimization** dialog,

- Select to perform buffer conditioning before experiment, or not.
- Enter Sample Volume.

Note: This will be the total volume in the reservoir + retentate holdup volume.

Note: The created optimization method will include a “chasing block” which will fill the system with sample without any dilution.

- Select number of crossflows
- Select Feed Parameter: Retentate Flow.

Note: The other options are DeltaP and Feed flow.

- Enter the retentate flows of the experiment plan.
- Enter the TMP settings of the plan.
- There is an option to select Retentate recovery after the run. The retentate loop will be emptied according to the procedure “Recovery with no flushes”, see Section 7.1.6.

UF Process Optimization

☒ Buffer Conditioning before Start

Fill step will use 26 ml extra volume, i.e. if you write 100 ml when the system will use 126 ml.

Sample Volume (36-350 ml)

Note: Sample volume is total retentate volume

Number of crossflows

Feed Parameter

Crossflow 1: ml/min (0-600 ml/min)

Crossflow 2: ml/min (0-600 ml/min)

Crossflow 3: ml/min (0-600 ml/min)

TMP Test Points

Number of Points

TMP Point 1 bar (0.0-5.2 bar)

TMP Point 2 bar (0.0-5.2 bar)

TMP Point 3 bar (0.0-5.2 bar)

TMP Point 4 bar (0.0-5.2 bar)

TMP Point 5 bar (0.0-5.2 bar)

☒ Retentate Recovery

-
- 6 Click **Next**.

When **Next** is clicked, a **Summary** of required solutions will be displayed.

Note: *An extra volume of sample is needed which is specified in the Summary page.*

- 7 To display the list of variables click **Finish**.

To edit a variable's value, click in the cell and change the value.

- 8 To edit a variable name, click **Edit Variable**.

- 9 Select a variable and edit.

Note: *If a variable's value is changed, it will affect the created method. A changed variable may also affect the calculated required solution volumes in the previous **Summary** dialog.*

Note: *We do recommend to not edit any variables, especially not the detail variables.*

- 10 To view the method, click the Text Editor icon.

The method is displayed as blocks.

- 11 To view and expand the method. Use the keyboard arrows.

- 12 To save this first method step, select **File:Save As** in the UNICORN Method Editor.

- 13 Browse for a folder, enter a method name and click **OK**.

6.4 Continuing the experiment

Two protein concentrations were included in the experiment plan. So, the procedure has to be repeated for the other protein concentration.

- 1 Run the method with the first protein concentration.
- 2 Clean the filter.
- 3 Run the method with the second protein concentration.

Note: *It may also be possible to perform this kind of experiment using the Scouting function in UNICORN. See UNICORN documentation.*

6.5 Evaluating results

Evaluate the results with the UNICORN Evaluation module, see *User Reference Manual - UNICORN 5.1 - Evaluation for Cross Flow Filtration*.

Example of results

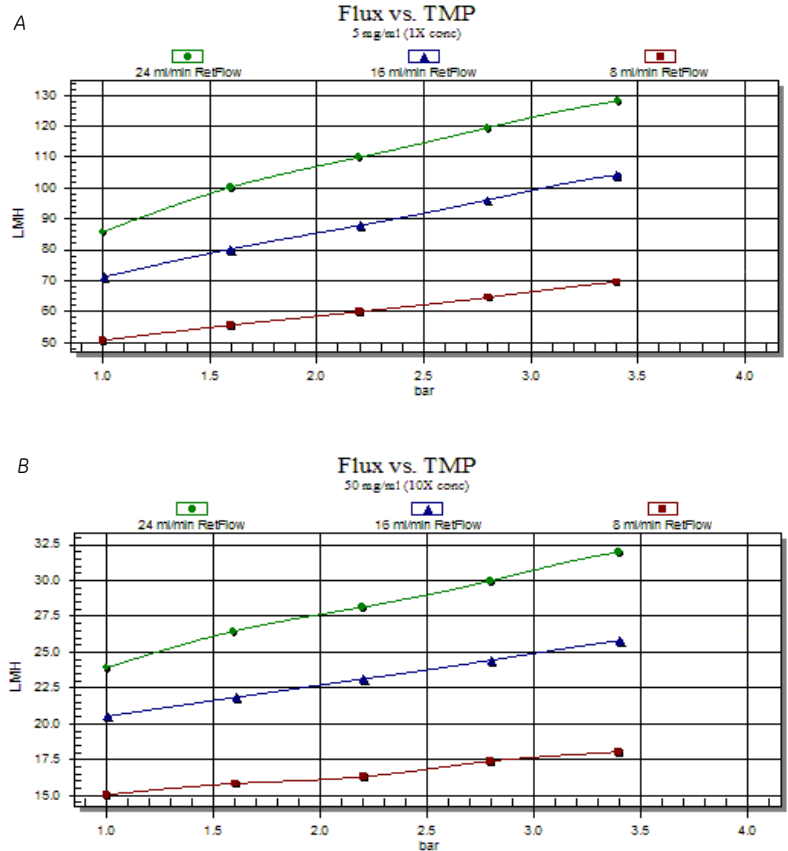


Fig 6-1. Evaluation of TMP-excursions runs with A: 5 mg/ml; B: 50 mg/ml Antibody samples.

Note: To be able to show several cross flows in a graph, chromatograms have to be bundled. See Section 11.3.2.



7 Creating Product methods using the Method Wizard

7.1 Ultrafiltration

7.1.1 Introduction

Concentration

The volume of sample in the reservoir is reduced. The product is retained at the retentate side of the membrane.

If the sample volume is larger than the reservoir volume, the reservoir can be continuously fed with sample solution (Fed Batch).

Diafiltration

Diafiltration is a filtration process used for the removal of smaller ionic solutes, whereby the feed solution is continuously filled up with a buffer. One buffer is removed and replaced with an alternative buffer (buffer exchange). The product is retained at the retentate side.

A buffer exchange is typically run after an concentrating step using the same filter as for the concentration step. So, the methods can be combined to one method.

If diafiltration is the single step and the sample volume is low, the hold-up volume on the retentate side will dilute the sample. Therefore, in this situation, we recommend to perform an initial concentration and enter a Concentration factor of "1" as endpoint. See Section 7.1.4, "Endpoint".

Product recovery after Concentration/Diafiltration

There are two alternatives to recover the product in the retentate:

- **No Recovery:** An option to select if the retentate volume will be drained manually.
- **Recovery:** The reservoir is first emptied through RVB-Out3. A defined number of flushes can then be selected and the retaining product will be emptied through RVB-Out1.

For descriptions of the Recovery procedures, see Section 7.1.7, Recovery section.

7.1.2 Basic settings dialog

Note: To get the Method Wizard for flat sheets, "Flat-sheet" has to be selected as component in System Setup. See Section 2.4.

Method Wizard for System: ÅKTAcrossflow1

Basic Settings

Method: Ultrafiltration

Filter List: Kwick Start 5 KD

Concentration/Diafiltration

Steps: ☒ Preproduct ☒ Product ☒ Postproduct

Flat Sheet (specification per filter)

Surface Area: 50 cm² (40-150 cm²)

Filter Hold-Up Volume: 1.4 ml (0.0-25.0 ml)

Feed Pressure Limit: 4.0 bar (0-5.2 bar)

TMP Limit: 4.0 bar (0-5.2 bar)

System setup

Number of filters: 1

Extra Tubing Volume: 0.0 ml (0.0-25.0 ml)

Reservoir Size: ☒ 350 ml ☐ 1100 ml

Tubing kit: ☒ Small ID (1.7 mm) ☐ Large ID (2.9 mm)

< Back Next > Finish Cancel Help Set Default

- In the **Method** list, select Ultrafiltration.

Basic Settings

Method: Ultrafiltration

Filter List: Kwick Start 5 KD

Steps: ☒ Preproduct ☒ Product ☒ Postproduct

- In the **Filter List**, available GE Healthcare filters are displayed. Select the used filter or select Other.

Basic Settings

Method: Ultrafiltration

Filter List: Kwick Start 5 KD

Steps: ☒ Preproduct ☒ Product ☒ Postproduct

- In the next list, select Concentration/Diafiltration.

Basic Settings

Method	Ultrafiltration
Filter List	Kwick Start 5 KD - 50 cm ²
	Concentration/Diafiltration
	Concentration/Diafiltration
	UF Process Optimization

- In the next area, select the type of process step.

Steps

☐ Preproduct ☒ Product ☐ Postproduct

- The **Flat Sheet (specification per filter)** area displays the recommended default values for the selected filter type. Accept or edit the values.

Flat Sheet (specification per filter)

Surface Area	50	cm ² (40-150 cm ²)
Filter Hold-Up Volume	1.4	ml (0.0-25.0 ml)
Feed Pressure Limit	4.0	bar (0-5.2 bar)
TMP Limit	4.0	bar (0-5.2 bar)

If "Other" filter type has been selected, enter Surface Area, Hold Up Volume, Feed pressure and TMP limits for the flat sheet. Information is usually available from the manufacturer.

Note: When using TMP in a method, a value close to "TMP limit" may lead to the run being paused. Always choose a lower working TMP than the "TMP limit".

- If several filters are assembled together in parallel, select in the **System setup** section the Number of filters (1-3). If one filter is used only, keep the default value "1".
- If other tubing, than standard, is used, calculate the extra tubing volume and enter the value in the "Extra Tubing Volume" box.

System setup

Number of filters	1
Extra Tubing Volume	0.0 ml (0.0-25.0 ml)

- Select Reservoir size and Tubing kit used in the recycle loop.

Reservoir Size

☒ 350 ml

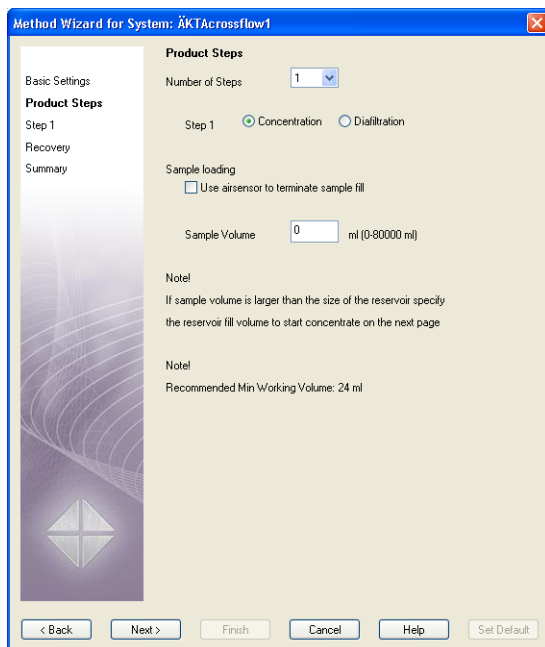
☐ 1100 ml

Tubing kit

☒ Small ID (1.7 mm)

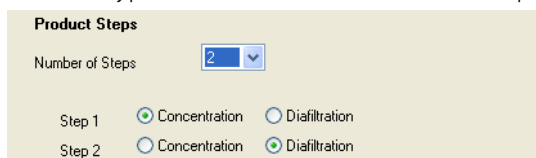
☐ Large ID (2.9 mm)

7.1.3 Product steps dialog

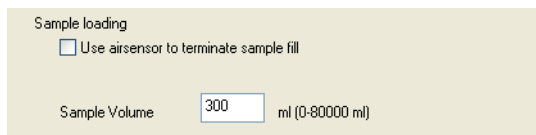


In the **Product Steps** dialog, select:

- Number of steps: 0-3.
- Select the type of ultrafiltration for the selected steps.



- Enter Sample Volume.



- If **Use airsensor to terminate sample fill** is selected, the air sensor connected to Transfer_Inlet 1 in the transfer block will stop sample loading when the sample container is empty.

Note: A Maximum Sample Volume has to be entered.

Sample loading

☒ Use airsensor to terminate sample fill

Maximum Sample Vol ml (0-80000 ml)

- **Fed Batch:** A volume larger than the reservoir can be selected. In this case, the Fed Batch function continuously fills the reservoir meanwhile permeate leaves the system during the concentration. This is possible when concentration is the first step. The initial volume in reservoir has to be defined on the next page.

Step 1 Concentration

Reservoir fill volume ml (5-350 ml)
to start concentrate

A Minimum working volume is displayed. The minimum working volume is the system holdup volume including filter, with an addition of a small volume in the reservoir. This volume depends on the reservoir size, tubing kit and filter volume. This is the lowest working volume that is recommended.

Note: Minimum working volume will vary with reservoir size and tubing kit used and is not the same as system holdup volume. To ensure process performance a smaller volume is added to the system holdup volume in the calculation of Minimum working volume. For information on system holdup volume, see *ÄKTAcrossflow Instrument Handbook*.

CAUTION! Do not use a volume below 50 ml in the **Large reservoir** as this carries a risk of getting air in the retentate.

7.1.4 Step 1 Concentration dialog

Method Wizard for System: ÅKTAcrossflow System 1

Step 1 Concentration

Basic Settings
Product Steps
Step 1
Step 2
Recovery
Summary

Feed Control: Retentate Flow

Retentate flow: 0 ml/min (0-600 ml/min)

TMP: 0.0 bar (0.0-5.2 bar)

Endpoint: OFF

Watch for Endpoint: OFF

☒ Retentate Volume: 26 ml (26-320 ml)
☐ Concentration Factor

< Back Next > Finish Cancel Help Set Default

Feed Control

- Select to perform TMP control with constant DeltaP, constant Feed Flow or constant Retentate Flow.

Feed Control: Retentate Flow

Retentate flow

Retentate Flow
Delta P
Feed Flow
Retentate Flow

- Enter the value for the selected constant parameter, for example Retentate Flow: 25 ml/min.
- Enter the desired TMP.

Note: When using TMP in a method, a value close to the "TMP limit" set in the Basic Settings dialog, may lead to the run being paused.

Feed Control: Retentate Flow

Retentate flow: 25 ml/min (0-600 ml/min)

TMP: 2.5 bar (0.0-5.2 bar)

Endpoint

- There are two kind of endpoints: “Watch for Endpoint” or “Retentate Volume/Concentration Factor”.
- The “Watch for” options are: “OFF”, “UV Greater than” and “UV Less than”.

- Enter a **Level** value for a selected “Watch for Endpoint”.
 - **Level:** The signal falls outside a specified value.
- If the first endpoint is selected, a Maximum endpoint has to be entered, Retentate Volume or Concentration factor. Select and enter a value.

CAUTION! Do not use a volume below 50 ml in the **Large reservoir** as this carries a risk of getting air in the retentate.

- If the “Watch for” option is set to OFF, the selected Retentate Volume or Concentration factor will be the only endpoint.
- For two endpoints, the endpoint of the process will be when one of the two endpoint conditions is met.
- Concentration factor:

Note: A Concentration factor between 0-50 can be entered. A value of 1 means no concentration. A value <1 means dilution. Values between 0-1 are used by the software to compensate for dilutions, for example due to the hold-up volume on the retentate side. This feature can be used when performing diafiltration of small volumes as a planned single step. See Section 7.1.1, “Diafiltration”.

7.1.5 Step 2 Diafiltration dialog

Note: If diafiltration of a small volume is planned, we recommend performing a concentration as an initial step. This is to avoid dilution of the sample due to the hold-up volume on the retentate side. For information, see Section 7.1.1, "Diafiltration" and Section 7.1.4, "Endpoint".

Method Wizard for System: ÄKTAcrossflow System 1

Step 2 Diafiltration

Basic Settings
Product Steps
Step 1
Step 2
Recovery
Summary

Feed Control: Retentate Flow

Retentate flow: 0 ml/min (0-600 ml/min)

TMP: 0.0 bar (0.0-5.2 bar)

Endpoint: OFF

Watch for Endpoint: OFF

☒ Permeate Volume: 20 ml (20-9999 ml)
☐ DF Exchange Factor

< Back Next > Finish Cancel Help Set Default

Feed Control

- Select to perform TMP control with constant DeltaP, constant Feed Flow or constant Retentate Flow.

Feed Control: Retentate Flow

Retentate flow

Retentate Flow
Delta P
Feed Flow
Retentate Flow

- Enter the value for the selected constant parameter, for example Retentate Flow: 25 ml/min.

- Enter the desired TMP.

Note: When using TMP in a method, a value close to the “TMP limit” set in the Basic Settings dialog, may lead to the run being paused.

Feed Control

Retentate Flow: 25 ml/min (0-600 ml/min)

TMP: 2.5 bar (0.0-5.2 bar)

Endpoint

- There are two kind of endpoints: “Watch for Endpoint” and “Permeate Volume/DF Exchange Factor”.

Endpoint

Watch for Endpoint: OFF

Permeate Volume: 0 ml (0-9999 ml)

DF Exchange Factor

- The “Watch for” options are shown in the screen below.

Endpoint

Watch for Endpoint: UV Less than

Level

Max Volume

Permeate Volume

DF Exchange Factor

- Enter parameters for the selected “Watch for Endpoint”.
 - Level:** The signal falls below a specified “Less than” value or is above a “Greater than” value.
 - Time Stable/Delta Base:** The signal is stable within the limits of the **Delta Base** value for the period specified by the minutes parameter (**Time Stable**).

Endpoint

Watch for Endpoint: Cond Less than and Stable Cond

Level: 20 mS/cm (0-999 mS/cm)

Time Stable: 1 min (0-9999 min)

Delta Base: 2 mS/cm (0-999 mS/cm)

- If the first endpoint is selected, a Max. endpoint has to be entered, Permeate Volume or Diafiltration(DF) Exchange Factor. Select and enter a value.

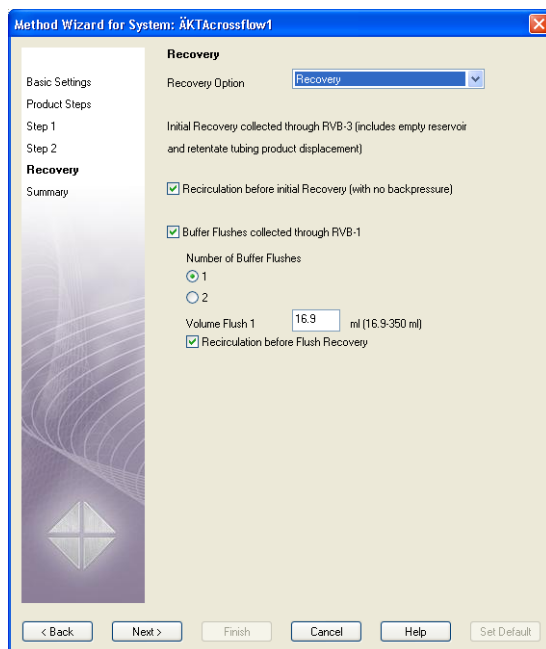
Max Endpoint

Permeate Volume

DF Exchange Factor: 10 (0-50)

- If the “Watch for” option is set to OFF, the selected Permeate Volume or Diafiltration(DF) Exchange Factor will be the only endpoint.
- For two endpoints, the endpoint of the process will be when one of the two endpoint conditions is met.

7.1.6 Recovery dialog



- In the **Recovery** dialog, there are two alternatives:
 - **No Recovery:** An option to select if the retentate volume will be drained manually.

Note: *If a retentate valve block of the old type is used by the system: To avoid siphoning, do not place the recovery vials too low.*

- **Recovery:** The reservoir is first emptied through RVB-Out3. A defined number of flushes can then be selected and the retaining product will be emptied through RVB-Out1.

For descriptions of the Recovery procedures, see Section 7.1.7, Recovery section.

No Recovery

- When this alternative is selected, the system will hold after the product steps. No postproduct steps are allowed in the method.

Recovery

Recovery Option No Recovery

Note! No Postproduct Steps are allowed when No Recovery is selected.
The system will hold when product steps have finished.

Note: If a retentate valve block of the old type is used by the system: To avoid siphoning, do not place the recovery vials too low.

Recovery

- Select whether or not to perform Recirculation before initial recovery. The recirculation, without pressure on the filter, will wash out product, e.g. in the gel layer, from the filter.
- If Buffer flushes are selected, enter number of flushes and flush volumes. Default volumes are dependent on filter hold-up volumes.
- If buffer flushes are not selected,
 - the reservoir will first be emptied through R-VB-Out3.
 - The reservoir will be filled with 5 ml buffer and the product between reservoir and RVB-Out3 will be emptied through RVB-Out3.
- There is also an option of performing recirculation between the flushes.

Recovery

Recovery Option Recovery

Initial Recovery collected through RVB-3 (includes empty reservoir and retentate tubing product displacement)

☒ Recirculation before initial Recovery (with no backpressure)

☒ Buffer Flushes collected through RVB-1

Number of Buffer Flushes

☒ 1 ☐ 2

Volume Flush 1 16.9 ml (16.9-350 ml)

☒ Recirculation before Flush Recovery

Note: The product will leave the system through R-VB-Out3 and the flushed volumes through R-VB-Out1.

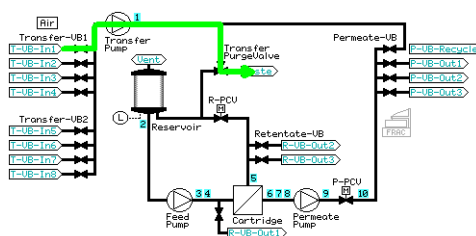
7.1.7 Visualized procedure of an Ultrafiltration method

Note: For information on the specific instructions, for example Constant Retentate Volume, see Chapter 13.

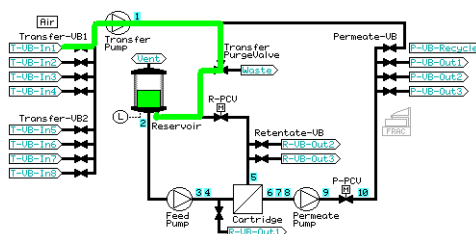
Sample filling

Example for a 100 ml sample volume:

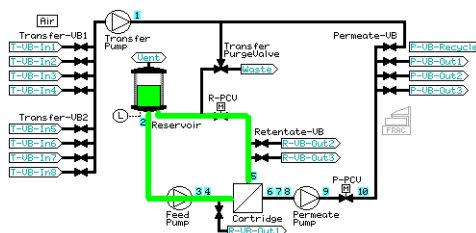
- 1 To prime the sample inlet tubing, Transfer pump fills the tubing with 6 ml sample from T-VB-In1(sample).
- 2 To prime the distance to Transfer Purge Valve-Waste, additional 10 ml is filled.



- 3 Reservoir is filled with 90 ml sample.

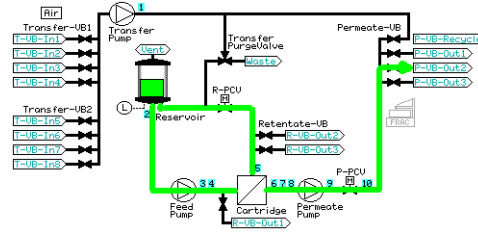


- 4 The last 10 ml of the sample is chased with buffer to the reservoir.
- 5 Recirculation on the retentate side.

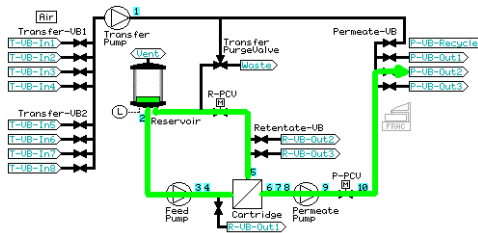


Concentration

- 1 Concentration starts with TMP control which activates the permeate pump and permeate valve block P-VB-Out2 is opened.



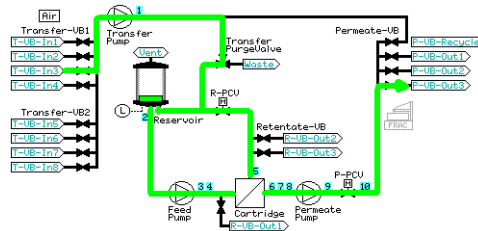
- 2 The concentration continues, still with P-VB-Out2 open, until the end point has been reached, specified Concentration factor, Retentate volume or Watch condition.



Diafiltration

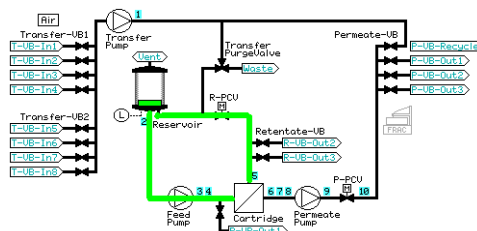
The volume in the reservoir is the volume specified as concentration end point, for example 50 ml.

- 1 At Constant Retentate Volume (50 ml) and TMP control, the permeate and transfer pumps are activated and P-VB-Out3 and T-VB-In3 are opened.
- 2 Liquid leaving the system through P-VB-Out3 is replaced with diafiltration buffer through T-VB-In3 until the end point has been reached, DF Exchange factor, Permeate volume or Watch for Endpoint (pH, conductivity or UV). If two diafiltration steps are performed, the second step will use buffer from T-VB-In4.

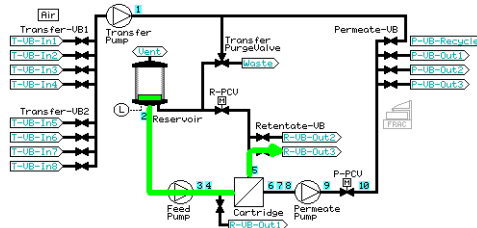


Product recovery: with no flushes

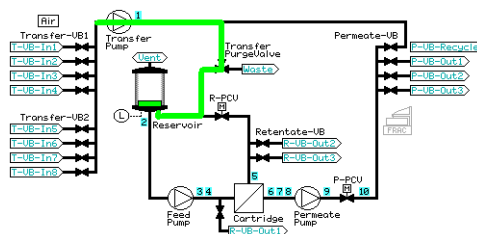
- Option to Recirculate before emptying the reservoir. Flowrates are the same as for the previous step.



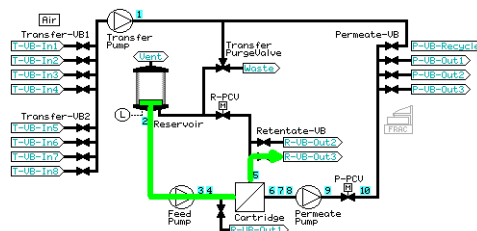
- The reservoir is emptied through R-VB-Out3 "Product".



- The reservoir is filled with 5 ml buffer from the Transfer valve block. Which inlet is dependent of the previous type of process, concentration, diafiltration 1 or diafiltration2.



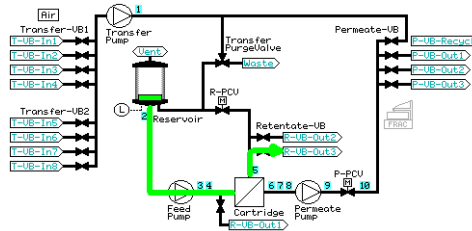
- The product between reservoir and R-VB-Out3 is chased with buffer and is emptied through R-VB-Out3



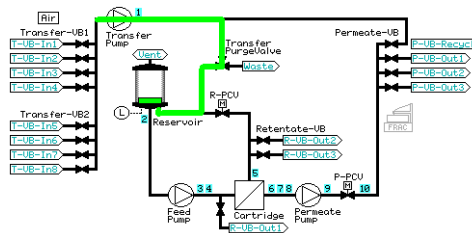
Recovery: with buffer flushes

In this example the retentate volume will be recovered with two buffer flushes of 16.9 ml each.

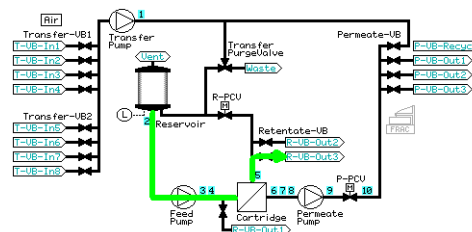
- 1 The reservoir is emptied through R-VB-Out3.



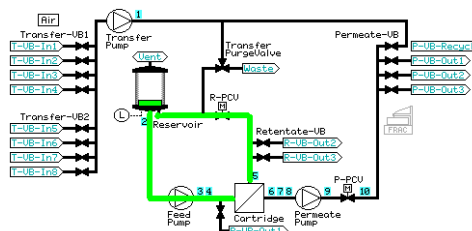
- 2 The reservoir is filled with 16.9 ml buffer according to the last product step.



- 3 Approx. 12 ml is emptied through R-VB-Out3. The product is chased with buffer through R-VB-Out3



- 4 Recirculation for five minutes.



- 5 5 ml through R-VB-Out1 is emptied.

-
- 6 The reservoir is filled with 16.9 ml buffer. The product is chased with approx. 10 ml buffer to R-VB-Out1. Recirculation for 5 minutes. Reservoir is emptied to R-VB-Out1.
 - 7 The reservoir is filled with 5 ml.
 - 8 At ConstantRetentateVolume, the retaining product between reservoir and R-VB-Out1, is chased with buffer and is emptied through R-VB-Out1.
 - 9 The rest of the system volume is emptied through waste.

7.2 Microfiltration

7.2.1 Introduction

Depending on the application, the product of interest will:

- stay in the retentate, or
- pass through the filter to the permeate side.

Cell harvesting/washing

In cell harvesting and cell washing, the product of interest (the cells) will stay in the retentate.

- A concentration reduces the volume of the cell solution.
- A diafiltration is run to wash the harvested cells.

Lysate clarification

After cell harvesting, mechanical disruption of the cells releases the product of interest from the cells and creates a lysate.

- In a concentration step, cells, cell debris or other insoluble matter are retained by the filter and the target product is passing the filter to the permeate.

Note: *In this step, the product leaves the system through P-VB-Out2.*

- A diafiltration step is then performed to flush the rest of the product of interest through the membrane.

Note: *In this step, the product leaves the system through P-VB-Out3.*

Product recovery after Concentration/Diafiltration

- For Lysate clarification, the target product is passing the filter to the permeate. The retentate can be directed to waste.
- If the product is in the retentate, there are two alternatives to recover the product:
 - No Recovery: An option to select if the retentate volume will be drained manually.

Note: *If a retentate valve block of the old type is used by the system: To avoid siphoning, do not place the recovery vials too low.*

- Recovery: The reservoir is first emptied through RVB-Out3. A defined number of flushes can then be selected and the retaining product will be emptied through RVB-Out1.

For descriptions of the Recovery procedures, see Section 7.1.7, Recovery section.

7.2.2 Basic settings dialog

Note: *To get the Method Wizard for hollow fibers, "Hollow fiber" has to be selected as component in System Setup. See Section 2.4.*

The screenshot shows the 'Method Wizard for System: ÅKTAcrossflow2' dialog box with the 'Basic Settings' tab selected. The left sidebar contains a tree view with 'Basic Settings' highlighted. The main area contains the following settings:

- Method:** Ultrafiltration/Cell Processing (dropdown)
- Filter Type:** A/M (dropdown)
- Filter List:** A/M 3 KD - 0.50 mm (dropdown)
- Steps:**
 - ☒ Preproduct
 - ☒ Product
 - ☒ Postproduct
- Hollow Fibre (specification per filter):**
 - Lumen Diameter:** 0.5 mm (0.10-10.00 mm)
 - Number of Fibers:** 12 (1-1000)
 - Surface Area:** 50 cm² (40-150 cm²)
 - Lumen Hold-Up Vol:** 1.17 ml (0.0-25.0 ml)
 - Feed Pressure Limit:** 4.3 bar (0.5-2 bar)
 - TMP Limit:** 3.0 bar (0.5-2 bar)
- System setup:**
 - Number of filters:** 1 (dropdown)
 - Extra Tubing Volume:** 0.0 ml (0.0-25.0 ml)
 - Reservoir Size:**
 - ☒ 350 ml
 - ☐ 1100 ml
 - Tubing kit:**
 - ☒ Small ID (1.7 mm)
 - ☐ Large ID (2.9 mm)

At the bottom, there are buttons: '< Back', 'Next >', 'Finish', 'Cancel', 'Help', and 'Set Default'.

- In the **Method** list, select Ultrafiltration/Cell Processing.

Basic Settings

Method: Ultrafiltration/Cell Processing

Filter Type: Calibrate level sensor
Installation Test
System Sanitization
Ultrafiltration/Cell Processing

Filter List: Ultrafiltration/Cell Processing

- Select Filter Type, AXM or AXH. AXM hollow fibers have a straight linear shape. AXH hollow fibers are curved into a loop.
- In the **Filter List**, available GE Healthcare filters are displayed. Select the used filter or select Other.

Basic Settings

Method: Ultrafiltration/Cell Processing

Filter Type: AXM

Filter List: AXM 3 KD - 0.50 mm

AXM 3 KD - 0.50 mm
AXM 10 KD - 0.50 mm
AXM 30 KD - 0.50 mm
AXM 100 KD - 0.50 mm
AXM 300 KD - 0.50 mm
AXM 500 KD - 0.50 mm
AXM 500 KD - 1.00 mm
AXM 750 KD - 1.00 mm
AXM 0.10 µm - 1.00 mm
AXM 0.20 µm - 1.00 mm
AXM 0.45 µm - 1.00 mm
AXM 0.65 µm - 0.75 mm
Other...

- Select the type of process step.

Steps

☐ Preproduct ☒ Product ☐ Postproduct

- The **Hollow Fibre (specification per filter)** area displays the recommended default values for the selected filter. Accept or edit the values.

Hollow Fibre (specification per filter)

Lumen Diameter	0.5	mm (0.10-10.00 mm)
Number of Fibers	12	(1-1000)
Surface Area	50	cm ² (40-150 cm ²)
Lumen Hold-Up Vol	1.17	ml (0.0-25.0 ml)
Feed Pressure Limit	4.3	bar (0-5.2 bar)
TMP Limit	3.0	bar (0-5.2 bar)

If "Other" filter has been selected, enter Lumen diameter, Number of fibers, Surface area, Lumen hold-up volume, Feed pressure limit and TMP limit for the hollow fiber. Information is usually available from the manufacturer.

Note: When using TMP in a method, a value close to "TMP limit" may lead to the run being paused. Always choose a lower working TMP than the "TMP limit".

- If several filters are assembled together in parallel, select in the **System setup** section the Number of filters (1-3). If only one filter is used, keep the default value “1”.
- If other tubing than standard is used, calculate the extra tubing volume and enter the value in the “Extra Tubing Volume” box.

System setup

Number of filters	1	
Extra Tubing Volume	0.0	ml (0.0-25.0 ml)

- Select Reservoir size and Tubing kit used in the recycle loop.

Reservoir Size

☒ 350 ml

☐ 1100 ml

Tubing kit

☒ Small ID (1.7 mm)

☐ Large ID (2.9 mm)

7.2.3 Product steps dialog

Method Wizard for System: ÄKTAcrossflow System 2

Product Steps

Number of Steps: 1

Step 1: ☒ Concentration ☐ Dialfiltration

☐ Load all Sample

Sample Volume: 0 ml (0-80000 ml)

Note! Recommended Min Working Volume: 30 ml

< Back Next > Finish Cancel Help Set Default

In the **Product Steps** dialog, select:

- Number of steps: 0-3.
- Select the type of ultrafiltration for the selected steps.

Product Steps

Number of Steps

Step 1 ☒ Concentration ☐ Diafiltration

Step 2 ☐ Concentration ☒ Diafiltration

- Enter Sample Volume.

Sample loading

☐ Use airsensor to terminate sample fill

Sample Volume ml (0-80000 ml)

- If **Use airsensor to terminate sample fill** is selected, the air sensor connected to Transfer_Inlet 1 in the transfer block, will stop sample loading when the sample container is empty.

Note: A Maximum Sample Volume has to be entered.

Sample loading

☒ Use airsensor to terminate sample fill

Maximum Sample Vol ml (0-80000 ml)

- **Fed Batch:** A volume larger than the reservoir can be selected. In this case, the Fed Batch function continuously fills the reservoir meanwhile permeate leaves the system during the concentration. This is possible when concentration is the first step.

The initial volume in reservoir has to be defined on the next page.

Step 1 Concentration

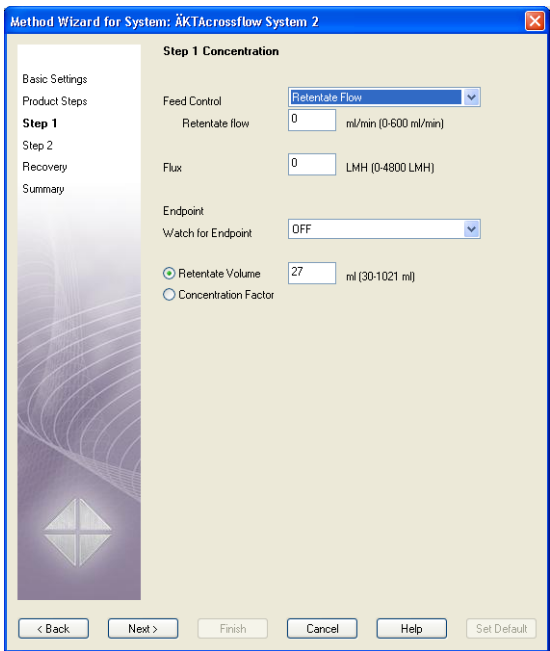
Reservoir fill volume ml (5-350 ml)
to start concentrate

A Minimum working volume is displayed. The minimum working volume is the system holdup volume including filter, with an addition of a small volume in the reservoir. This volume depends on the reservoir size, tubing kit and filter volume. This is the lowest working volume that is recommended.

Note: Minimum working volume will vary with reservoir size and tubing kit used and is not the same as system holdup volume. To ensure process performance a smaller volume is added to the system holdup volume in the calculation of Minimum working volume. For information on system holdup volume, see ÄKTAcrossflow Instrument Handbook

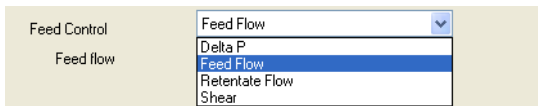
CAUTION! Do not use a volume below 50 ml in the **Large reservoir** as this carries a risk of getting air in the retentate.

7.2.4 Step 1 Concentration dialog



Feed Control

- Select to perform Feed control with constant DeltaP, constant Feed Flow, constant Retentate Flow or constant Shear.



- Enter a value for the selected constant parameter, for example Feedflow: 500 ml/min.

Feed Control	Feed Flow
Feed flow	500 ml/min (0-600 ml/min)

Control Mode

- Select to have Flux or TMP as Control mode.

Control Mode	Flux
Flux	Flux TMP

- Enter a value for the selected control mode, for example Flux 45 LMH.

Control Mode	Flux
Flux	45 LMH (0-4800 LMH)

Endpoint

- There are two kind of endpoints: "Watch for Endpoint" or "Retentate Volume/Concentration Factor".
- The "Watch for" options are: "OFF", "UV Greater than" and "UV Less than".

Endpoint	
Watch for Endpoint	UV Less than
Level	OFF UV Greater than UV Less than
Max Volume	UV Less than

- Enter a **Level** value for a selected "Watch for Endpoint".
 - Level:** The signal falls outside a specified value.
- If the first endpoint is selected, a Max. endpoint has to be entered, Retentate Volume or Concentration factor. Select and enter a value.

Endpoint	
Watch for Endpoint	UV Greater than
Level	10 mAU (-7000-7000 mAU)
Max Endpoint	
☒ Retentate Volume	100 ml (30-1021 ml)
☐ Concentration Factor	

- If the "Watch for" option is set to OFF, the selected Retentate Volume or Concentration factor will be the only endpoint.

CAUTION! Do not use a volume below 50 ml in the **Large reservoir** as this carries a risk of getting air in the retentate.

- For two endpoints, the endpoint of the process will be when one of the two endpoint conditions is met.
- Concentration factor:

Max Endpoint

☐ Retentate Volume

☒ Concentration Factor (0-50)

Note: A Concentration factor between 0-50 can be entered. A value of 1 means no concentration. A value <1 means dilution. Values between 0-1 are used by the software to compensate for dilutions, for example due to the hold-up volume on the retentate side. This feature can be used when performing diafiltration of small volumes as a planned single step. See Section 7.1.1, "Diafiltration".

7.2.5 Step 2 Diafiltration dialog

Method Wizard for System: ÄKTAcrossflow System 2

Step 2 Diafiltration

Basic Settings

Product Steps

Step 1

Step 2

Recovery

Summary

Feed Control: Retentate Flow

Retentate flow: 0 ml/min (0-600 ml/min)

Flux: 0 LMH (0-4800 LMH)

Endpoint

Watch for Endpoint: OFF

☒ Permeate Volume: 20 ml (20-9999 ml)

☐ DF Exchange Factor

< Back Next > Finish Cancel Help Set Default

Feed Control

- Select to perform Feed control with constant DeltaP, constant Feed Flow, constant Retentate Flow or constant Shear.

Feed Control

Feed flow

Feed Flow

Delta P

Retentate Flow

Shear

- Enter a value for the selected constant parameter, for example Feedflow: 500 ml/min.

Feed Control	Feed Flow	
Feed flow	500	ml/min (0-600 ml/min)

Control Mode

- Select to have Flux or TMP as Control mode.

Control Mode	Flux
Flux	Flux TMP

- Enter a value for the selected control mode, for example Flux 45 LMH.

Control Mode	Flux
Flux	45 LMH (0-4800 LMH)

Endpoint

- There are two kind of endpoints: "Watch for Endpoint" and "Permeate Volume/Diafiltration(DF) Exchange Factor".

Endpoint	
Watch for Endpoint	OFF
☒ Permeate Volume	0 ml (0-9999 ml)
☐ DF Exchange Factor	

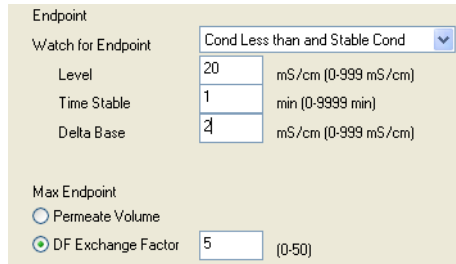
- The "Watch for" options are shown in the screen below.

Endpoint	
Watch for Endpoint	UV Less than
Level	Cond Greater than and Stable Cond Cond Less than and Stable Cond OFF pH Greater than and Stable pH pH Less than and Stable pH UV Greater than UV Less than
Max Volume	
☒ Permeate Volume	
☐ DF Exchange Factor	

- Enter parameters for the selected "Watch for Endpoint".
 - Level:** The signal falls below a specified value.
 - Time Stable/Delta Base:** The signal is stable within the limits of the **Delta Base** value for the period specified by the minutes parameter (**Time Stable**).

Endpoint	
Watch for Endpoint	Cond Less than and Stable Cond
Level	20 mS/cm (0-999 mS/cm)
Time Stable	1 min (0-9999 min)
Delta Base	2 mS/cm (0-999 mS/cm)

- If the first endpoint is selected, a Max. endpoint has to be entered, Permeate Volume or Diafiltration(DF) Exchange Factor. Select and enter a value.



Endpoint

Watch for Endpoint: Cond Less than and Stable Cond

Level: 20 mS/cm (0-999 mS/cm)

Time Stable: 1 min (0-9999 min)

Delta Base: 2 mS/cm (0-999 mS/cm)

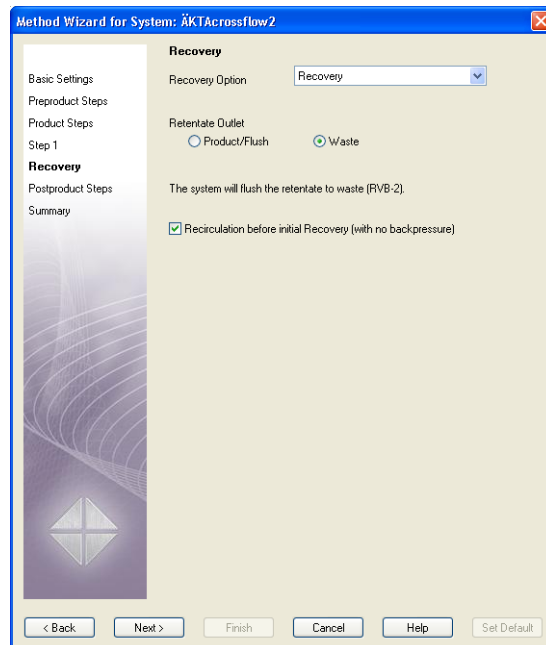
Max Endpoint

☐ Permeate Volume

☒ DF Exchange Factor: 5 (0-50)

- If the “Watch for” option is set to OFF, the selected Permeate Volume or Diafiltration(DF) Exchange Factor will be the only endpoint.
- For two endpoints, the endpoint of the process will be when one of the two endpoint conditions is met.

7.2.6 Recovery dialog



Method Wizard for System: ÅKTAcrossflow2

Recovery

Recovery Option: Recovery

Retentate Outlet: ☐ Product/Flush ☒ Waste

The system will flush the retentate to waste (FVB-2).

☒ Recirculation before initial Recovery (with no backpressure)

< Back Next > Finish Cancel Help Set Default

Recovery of permeate

- For Lysate clarification, the target product is passing the filter to the permeate. During the concentration step, through P-VB-Out2 and during the diafiltration step, through P-VB-Out3. The retentate can be directed to waste (R-VB-Out-2) by selecting **Waste**.

The screenshot shows a dialog box titled "Recovery". It contains a "Recovery Option" dropdown menu set to "Recovery". Below it, the "Retentate Outlet" section has two radio buttons: "Product/Flush" (unselected) and "Waste" (selected). A text box below the radio buttons states: "The system will flush the retentate to waste (RVB-2).". At the bottom, there is a checked checkbox labeled "Recirculation before initial Recovery (with no backpressure)".

Note: To clean the retentate side, it may be recommended to select "Recirculation before initial Recovery...".

Recovery of retentate

If the product is in the retentate as for Cell harvesting/ Cell washing, there are two alternatives to recover the product:

- No Recovery:** An option to select if the retentate volume will be drained manually.

Note: If a retentate valve block of the old type is used by the system: To avoid siphoning, do not place the recovery vials too low.

- Recovery:** The reservoir is first emptied through RVB-Out3. A defined number of flushes can then be selected and the retaining product will be emptied through RVB-Out1.

For descriptions of the Recovery procedures, see Section 7.1.7, Recovery section.

No Recovery

- When this alternative is selected, the system will hold after the product steps. No postproduct steps are allowed in the method.

The screenshot shows a dialog box titled "Recovery". It contains a "Recovery Option" dropdown menu set to "No Recovery". Below it, a text box states: "Note! No Postproduct Steps are allowed when No Recovery is selected. The system will hold when product steps have finished."

Note: If a retentate valve block of the old type is used by the system: To avoid siphoning, do not place the recovery vials too low.

Recovery

- Select to perform Recirculation before initial recovery, or not. The recirculation will wash out product, e.g. in the gel layer, from the filter.
- If Buffer flushes are selected, enter number of flushes and flush volumes. Default volumes are dependent on filter's hold-up volumes.
- If buffer flushes are not selected,
 - the reservoir will first be emptied through R-VB-Out3.
 - The reservoir will be filled with 5 ml buffer and the product between reservoir and RVB-Out3 will be emptied through RVB-Out3.
- There is also an option to perform recirculation between the flushes.

Recovery

Recovery Option Recovery

Initial Recovery collected through RVB-3 (includes empty reservoir and retentate tubing product displacement)

☒ Recirculation before initial Recovery (with no backpressure)

☒ Buffer Flushes collected through RVB-1

Number of Buffer Flushes

☒ 1

☐ 2

Volume Flush 1 ml (16.9-350 ml)

☒ Recirculation before Flush Recovery

Note: The product will leave the system through R-VB-Out3 and the flushed volumes through R-VB-Out1.



8 Creating Postproduct methods using the Method Wizard

8.1 Introduction

The following method steps in this order are recommended to run after a cross flow filtration:

- 1 Buffer or Water Flush: Flushes out remains of protein/cells before the system is treated with NaOH. The procedure is the same as for Water flush. The first block is "Preparing the system and reservoir". See Section 5.4.1, Section 5.2.3 and Section 5.4.5.
- 2 Filter CIP (Clean-in-place): For a description, see Section 5.2.2 and Section 5.4.4. The first block is "Preparing the system and reservoir". See Section 5.4.1.
- 3 Water Flush: For a description, see Section 5.2.3 and Section 5.4.5. The first block is "Preparing the system and reservoir". See Section 5.4.1.
- 4 Water Flux Test: For a description, see Section 5.2.4 and Section 5.4.6
- 5 Filter Storage Solution: The liquid in the filter is exchanged to an appropriate storage solution. For information on recommended storage solution, see enclosed filter instruction from manufacturer. The procedure is the same as for "Buffer conditioning", see Section 5.2.5 and Section 5.4.7.

Note: *A recommended storage solution for the system is 20% Ethanol. To exchange solution in the system, perform the Sanitization method and use 20% Ethanol as liquid. It is important to use a three-way-connector instead of a filter. See Section 10.1.*

Note: *If the system needs more intensive cleaning, it may also be necessary to perform a System sanitization. See Section 10.1.*

8.2 Postproduct methods: Method Wizard dialogs

8.2.1 Basic settings: Flat sheets

Note: To get the Method Wizard for flat sheets, "Flat-sheet" has to be selected as component in System Setup. See Section 2.4.

- In the **Method** list, select Ultrafiltration.

- In the **Filter List**, available GE Healthcare filters are displayed. Select the used filter or select Other.

- In the next list, select Concentration/Diafiltration.

Basic Settings

Method	Ultrafiltration
Filter List	Kwick Start 5 KD - 50 cm ²
	Concentration/Diafiltration
	Concentration/Diafiltration
	UF Process Optimization

- In the next area, select the type of process step.

Steps

☐ Preproduct ☐ Product ☒ Postproduct

- The **Flat Sheet (specification per filter)** area displays the recommended default values for the selected filter type. Accept or edit the values.

Flat Sheet (specification per filter)

Surface Area	50	cm ² (40-150 cm ²)
Filter Hold-Up Volume	1.4	ml (0.0-25.0 ml)
Feed Pressure Limit	4.0	bar (0-5.2 bar)
TMP Limit	4.0	bar (0-5.2 bar)

If "Other" filter type has been selected, enter Surface Area, Hold Up Volume, Feed pressure and TMP limits for the flat sheet. Information is usually available from the manufacturer.

Note: When using TMP in a method, a value close to "TMP limit" may lead to the run being paused. Always choose a lower working TMP than the "TMP limit".

- If several filters are assembled together in parallel, select in the **System setup** section the Number of filters (1-3). If only one filter is used, keep the default value "1".
- If other tubing, than standard, is used, calculate the extra tubing volume and enter the value in the "Extra Tubing Volume" box.

System setup

Number of filters	1
Extra Tubing Volume	0.0 ml (0.0-25.0 ml)

- Select Reservoir size and Tubing kit used in the recycle loop.

Reservoir Size

☒ 350 ml

☐ 1100 ml

Tubing kit

☒ Small ID (1.7 mm)

☐ Large ID (2.9 mm)

8.2.2 Basic settings: Hollow fibers

Note: To get the Method Wizard for hollow fibers, "Hollow fiber" has to be selected as component in System Setup. See Section 2.4.

Method Wizard for System: ÅKTAcrossflow2

Basic Settings

Method: Ultrafiltration/Cell Processing

Filter Type: AXM

Filter List: AXM 3 KD - 0.50 mm

Steps:

☒ Preproduct ☒ Product ☒ Postproduct

Hollow Fibre (specification per filter)

Lumen Diameter: 0.5 mm (0.10-10.00 mm)

Number of Fibers: 12 (1-1000)

Surface Area: 50 cm² (40-150 cm²)

Lumen Hold-Up Vol: 1.17 ml (0.0-25.0 ml)

Feed Pressure Limit: 4.3 bar (0-5.2 bar)

TMP Limit: 3.0 bar (0-5.2 bar)

System setup

Number of filters: 1

Extra Tubing Volume: 0.0 ml (0.0-25.0 ml)

Reservoir Size:

☒ 350 ml

☐ 1100 ml

Tubing kit:

☒ Small ID (1.7 mm)

☐ Large ID (2.9 mm)

< Back Next > Finish Cancel Help Set Default

- In the **Method** list, select Ultrafiltration/Cell Processing.

Basic Settings

Method: Ultrafiltration/Cell Processing

Filter Type: Calibrate level sensor
Installation Test
System Sanitization
Ultrafiltration/Cell Processing

Filter List: Ultrafiltration/Cell Processing

- Select Filter Type, AXM or AXH. AXM hollow fibers have a straight linear shape. AXH hollow fibers are curved into a loop.

- In the **Filter List**, available GE Healthcare filters are displayed. Select the used filter or select Other.

Basic Settings

Method: Ultrafiltration/Cell Processing

Filter Type: A×M

Filter List: A×M 3 KD - 0.50 mm

- A×M 3 KD - 0.50 mm
- A×M 10 KD - 0.50 mm
- A×M 30 KD - 0.50 mm
- A×M 100 KD - 0.50 mm
- A×M 300 KD - 0.50 mm
- A×M 500 KD - 0.50 mm
- A×M 500 KD - 1.00 mm
- A×M 750 KD - 1.00 mm
- A×M 0.10 µm - 1.00 mm
- A×M 0.20 µm - 1.00 mm
- A×M 0.45 µm - 1.00 mm
- A×M 0.65 µm - 0.75 mm
- Other...

- Select the type of process step.

Steps

☐ Preproduct ☐ Product ☒ Postproduct

- The **Hollow Fibre (specification per filter)** area displays the recommended default values for the selected filter. Accept or edit the values.

Hollow Fibre (specification per filter)

Lumen Diameter	0.5	mm (0.10-10.00 mm)
Number of Fibers	12	(1-1000)
Surface Area	50	cm ² (40-150 cm ²)
Lumen Hold-Up Vol	1.17	ml (0.0-25.0 ml)
Feed Pressure Limit	4.3	bar (0-5.2 bar)
TMP Limit	3.0	bar (0-5.2 bar)

If “Other” filter has been selected, enter Lumen diameter, Number of fibers, Surface area, Lumen hold-up volume, Feed pressure limit and TMP limit for the hollow fiber. Information is usually available from the manufacturer.

Note: When using TMP in a method, a value close to “TMP limit” may lead to the run being paused. Always choose a lower working TMP than the “TMP limit”.

- If several filters are assembled together in parallel, select in the **System setup** section the Number of filters (1-3). If only one filter is used, keep the default value “1”.
- If other tubing than standard is used, calculate the extra tubing volume and enter the value in the “Extra Tubing Volume” box.

System setup

Number of filters: 1

Extra Tubing Volume: 0.0 ml (0.0-25.0 ml)

- Select Reservoir size and Tubing kit used in the recycle loop.

Reservoir Size

☒ 350 ml

☐ 1100 ml

Tubing kit

☒ Small ID (1.7 mm)

☐ Large ID (2.9 mm)

8.2.3 Postproduct setup

Method Wizard for System: ÅKTAcrossflow1

Basic Settings

Postproduct Steps

Summary

☒ Flush

☒ Conditioning Buffer ☐ Water

☒ Filter CIP

☒ CIP 1

Length of Time min (0-9999 min)

☐ CIP 2

☒ Water Flush

☒ Water Flux Test

☒ TMP bar (0-5.2 bar)

☐ NFF

☐ Filter Storage Solution

< Back Next > Finish Cancel Help Set Default

Flush:

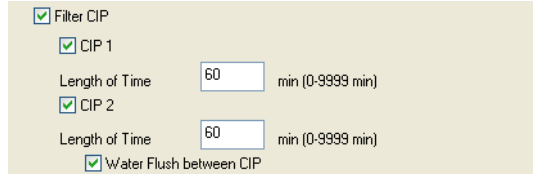
- To include a Flush in the method, check the **Flush** box and select to perform the flush with conditioning buffer or water.

☒ Flush

☒ Conditioning Buffer ☐ Water

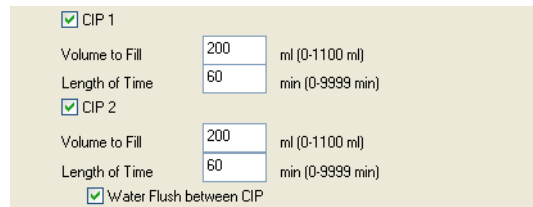
Filter CIP

- To include a Filter CIP, check the **Filter CIP** box.
- There is an option to select two CIP with a optional Water flush between.
- The circulation time of CIP solution is entered in the **Length of Time** boxes.



☒ Filter CIP
☒ CIP 1
 Length of Time min (0-9999 min)
☒ CIP 2
 Length of Time min (0-9999 min)
☒ Water Flush between CIP

- If the large reservoir is used, "Volume to Fill" has to be entered.



☒ CIP 1
 Volume to Fill ml (0-1100 ml)
 Length of Time min (0-9999 min)
☒ CIP 2
 Volume to Fill ml (0-1100 ml)
 Length of Time min (0-9999 min)
☒ Water Flush between CIP

Water flush

- To include a Water flush in the method, check the **Water Flush** box.

Note: After a Filter CIP, we recommend running a water flush.

Note: If a Water Flux test will be performed, Water Flush should be performed before the test.



☒ Water Flush

Water Flux test

- To include a Water Flux test in the method, check the **Water Flux Test** box.
- Select to use TMP or **NFF (Normal Flow Filtration)** as control mode. NFF is default for hollow fibers with cut off 0.1 µm and larger.
- For TMP as control mode:
 - Enter a TMP value for the test.

Note: When comparing status of a filter as a function of time and number of experiments, it is important to have performed the same type of method, (i.e. same TMP value).

Water Flux ☒

Water Flux Test ☒

☒ TMP bar (0-5.2 bar)

☐ NFF

- For NFF as control mode:
 - Select to perform Feed control for NFF with FeedFlow or FeedPressure.

Water Flux ☒

Water Flux Test ☒

☐ TMP

☒ NFF Feed Control for NFF

bar

- Enter a value for the selected Feed control.

Water Flux ☒

Water Flux Test ☒

☐ TMP

☒ NFF Feed Control for NFF

ml/min (0-200 ml/min)

Filter Storage Solution

- The liquid in the filter is exchanged to an appropriate storage solution. For information on recommended storage solution, see enclosed filter instruction from manufacturer. The procedure is the same as for “Buffer conditioning”, see Section 5.2.5 and Section 5.4.7.
- To include the step in the method, check the **Filter Storage Solution** box.

☒ Filter Storage Solution

Note: *A recommended storage solution for the system is 20% Ethanol. To exchange solution in the system, perform the Sanitization method and use 20% Ethanol as liquid. It is important to use a three-way-connector instead of a filter. See Section 10.1.*



9 Running ÄKTAcrossflow methods

9.1 Final preparation

This section describes the final preparations that should be done before starting a run.

The Summary page created by the Method Wizard will list calculated required volumes and corresponding inlet positions.

9.1.1 Solutions

- 1 Immerse the ends of the solution inlet tubing in the appropriate solution containers.
- 2 Check that there are sufficient solution volumes available.
- 3 Set ResVolTotalizer to zero in **UNICORN:System Control**.

Note: Use Milli-Q water, or equivalent, when preparing solutions and buffers.

9.1.2 Sample

- 1 Put the ends of the sample inlet tubing in the appropriate sample containers.
- 2 Check that there is sufficient sample available.

9.1.3 Waste

- 1 Check that following outlet tubing are placed in waste containers:
 - Transfer Purge Valve-Waste.
 - R-VB-Out2: Retentate Waste valve.
 - P-VB-Out1: Permeate Waste valve.
- 2 Check that the waste containers are not full and will accept the volume diverted to it during the run.

9.1.4 Filter

- 1 Check that the correct filter is assembled in a correct way.
- 2 Ensure the filter is clean and has acceptable quality. Appropriate test is Water flux test.

9.1.5 Calibration

Calibrate the pH electrode, conductivity meter and pressure sensors, if required. Refer to *ÄKTAcrossflow Instrument Handbook*. The Level sensor should be calibrated before every run. See Section 2.6.

9.1.6 WARNINGS and CAUTIONS

CIP method

When running a method using a CIP solution containing sodium hydroxide (NaOH):



WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective lab coat.

CAUTION! Always make sure that the filters and system components are compatible with sodium hydroxide at the concentration, time and temperature used.

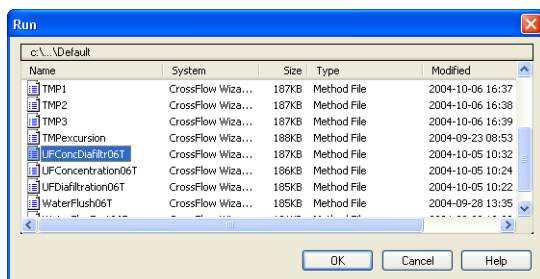
General



WARNING! Do not operate the ÄKTAcrossflow system at pressures above the specified maximum pressure (5.2 bar).

9.2 Starting a run

- 1 In the **System Control** module, select **File:Run**.
- 2 Select the method to start. Click **OK**.



A Start Protocol appears consisting of a number of dialog boxes.

- 3 On the **Variables** page, it is possible to fine-tune the method before proceeding

Note: We do recommend to not edit any variables, especially not the detail variables.

The Variables dialog box contains a table with the following data:

Block	Variable	Value	Range
PID_and_filter_block	P_Parameter_FeedPress	0.005	0.001 - 1000.000
	I_Parameter_FeedPress	150.000	0.000 - 10000.000
	P_Parameter_DeltaP	0.100	0.001 - 1000.000
	I_Parameter_DeltaP	10.000	0.000 - 10000.000
	P_Parameter_EmptyFeedPress	0.005	0.001 - 1000.000
	I_Parameter_EmptyFeedPress	150.000	0.000 - 10000.000
	P_Parameter_PUF_Perm	0.001	0.001 - 1000.000
	I_Parameter_PUF_Perm	60.000	0.000 - 10000.000
	P_Parameter_Flux_Ret	0.200	0.001 - 1000.000
	I_Parameter_Flux_Ret	50.000	0.000 - 10000.000
Empty_Reservoir_Completely	P_Parameter_TMP_Perm	0.001	0.001 - 1000.000
	I_Parameter_TMP_Perm	25.000	0.000 - 10000.000
Start_Settings_FS	Ret_Vol (ml)	20.00	0.00 - 5000.00
RetFlow_Step1	Filter_Area (cm²)	40.0	1.0 - 1000.0
TMP_Step1	Ret_Flow_Step1 (ml/min)	25.0	0.0 - 600.0
	TMP_Step_1 (bar)	2.50	0.00 - 5.20
Step1_Conc_G	Conc_Factor_Step1Cont	50.00	0.00 - 50.00

Below the table, there are three checkboxes:

- ☐ Show details
- ☐ Show unused variables
- ☒ Display tooltip for extended variable cells

At the bottom right, there is a **Help** button. At the bottom center, there are **< Back** and **Next >** buttons. At the bottom right, there is a **Cancel** button.

- 4 Click **Next** or **Back** to go through the dialog boxes and add the information that is required as well as your own comments.

Note: This list will also be displayed in **Method Notes** in the **Run Setup** dialog

Note: Incorrect information is shown in the **Method Duration** tab in the **Method Information** dialog. Instead, use the information shown in the **Summary** dialog in the Method Wizard. The Summary information is also displayed in the Method Notes tab in the Start protocol.

- Click the **START** button in the **Result Name** dialog box. This will initiate the method run.

9.3 Instant Run

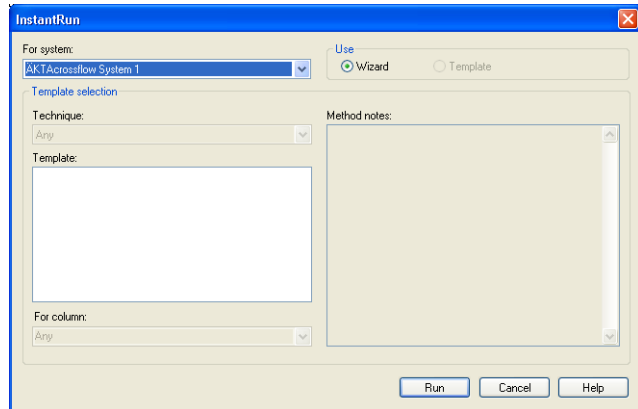
This instruction describes the easiest way to create a method, run the system and generate results. The procedure is based on an Instant run.

The use of Instant Run can be recommended when methods do not need to be saved, for example when running an Installation test or System sanitization.

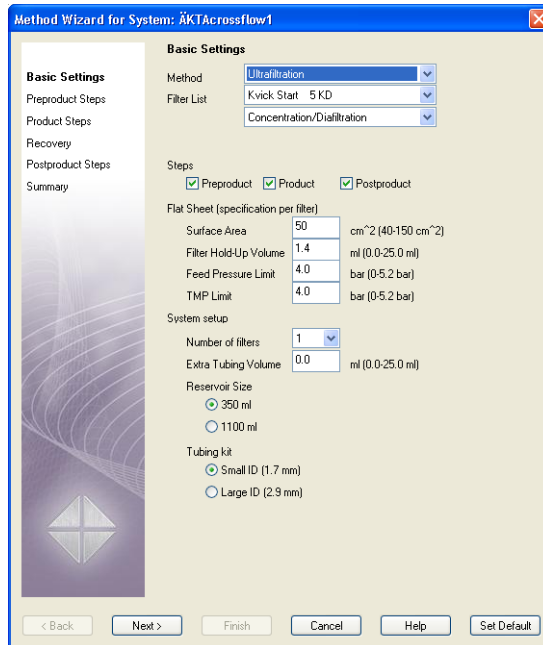


- Click the Instant run icon in the **UNICORN Manager** module, or select **File:Instant Run**.
- In the **Instant Run** dialog,
 - select to use **Wizard**.
 - Select a System.

- Click the **Run** button.



The **Method Wizard** is opened in the **System Control** module.



- Go through all selections on the **Method Wizard** pages. Click the **Next** button to proceed through several pages.

- 4 Click the **Run** button on the last page.

Method Wizard for System: ÄKTAcrossflow System 1

Summary

Basic Settings
Preproduct Steps
Product Steps
Step 1
Recovery
Postproduct Steps
Summary

System: ÄKTAcrossflow System 1

Inlet number	Definition	Volume(max)
1	Sample	40 ml
2	Conditioning buffer	1220 ml
3	Dialfiltration buffer 1	See note
4	Dialfiltration buffer 2	See note
5	Water	2630 ml
6	CIP solution 1	1040 ml
7	CIP solution 2	0 ml
8	Storage Solution	0 ml

Retentate out	Definition
1	Flush
2	Waste
3	Product

Permeate out	Definition
1	Waste
2	Conc
3	DF

Note 1
Please note that this info will be lost after "Finish" is executed. Print this page in order to keep this information.

Note 2
The DF volumes are not estimated here.

Print

< Back Next > Run Cancel Help Set Default

- 5 Verify the method on the **Variables** page

Note: We do recommend to not edit any variables, especially not the detail variables.

Variables

Block	Variable	Value	Range
Start_Settings_FS	Filter_Area (cm ²)	40.0	1.0 - 1000.0
PID_and_filter_block	P_Parameter_FeedPress	0.005	0.001 - 1000.000
	L_Parameter_FeedPress	150.000	0.000 - 10000.000
	P_Parameter_DeltaP	0.100	0.001 - 1000.000
	L_Parameter_DeltaP	10.000	0.000 - 10000.000
	P_Parameter_EmptyFeedPress	0.005	0.001 - 1000.000
	L_Parameter_EmptyFeedPress	150.000	0.000 - 10000.000
	P_Parameter_PUF_Perm	0.001	0.001 - 1000.000
	L_Parameter_PUF_Perm	60.000	0.000 - 10000.000
	P_Parameter_Flux_Ret	0.200	0.001 - 1000.000
	L_Parameter_Flux_Ret	50.000	0.000 - 10000.000
	P_Parameter_TMP_Perm	0.001	0.001 - 1000.000
	L_Parameter_TMP_Perm	25.000	0.000 - 10000.000
Empty_Reservoir_Completely	Ret_Vol (ml)	17.00	0.00 - 5000.00
RetFlow_Step1	Ret_Flow_Step1 (ml/min)	0.0	0.0 - 600.0
TMP_Step1	TMP_Step_1 (bar)	0.00	0.00 - 5.20
Flush_Vol	Flush_Volume (ml)	400.00	0.00 - 99999.00

☐ Show details
☐ Show unused variables
☒ Display tooltip for extended variable cells

Help

< Back Next > Cancel

- 6 Click the **Next** button to proceed through several pages.

- 7 Select Evaluation procedure, if appropriate.
- 8 Click the **Start** button on the last page. The Run starts.

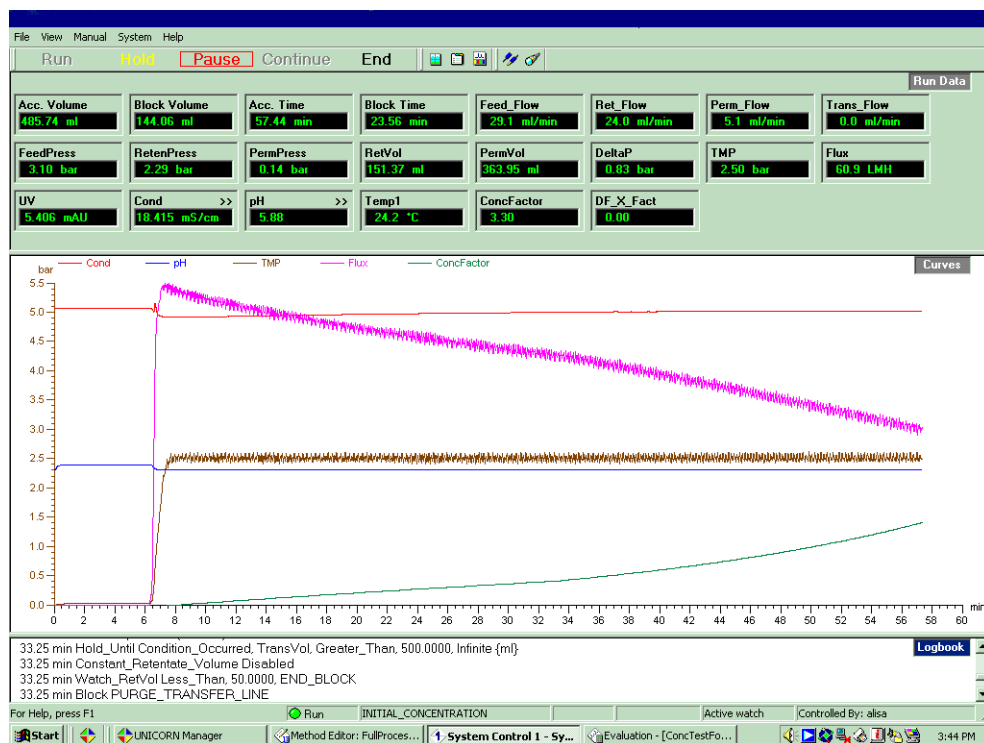
The screenshot shows a 'Result Name' dialog box with a blue title bar and a close button (X) in the top right corner. The dialog is divided into several sections:

- Run info:** Contains three text fields: 'Date' with the value '2005-01-26 16:31:10', 'User' with the value 'default', and 'Method' with the value 'C:\...\Wizard Generated.m03'.
- Result:** Contains two checkboxes: 'No result' (unchecked) and 'Add unique identifier to result name' (unchecked). Below these are three text fields: 'Directory' with the value 'Home' and a 'Browse...' button to its right; 'Scouting subdirectory' (empty); and 'Name' with the value 'Wizard Generated001'.
- Batch ID:** Contains a single empty text field.

At the bottom right of the dialog is a 'Help' button. At the very bottom of the dialog are four navigation buttons: '< Back', 'Next >', 'START' (highlighted in yellow), and 'Cancel'.

9.4 During the run

The progress of the method being used can be viewed in detail in UNICORN. The **System Control** module displays the current status of ÄKTAcrossflow and displays up to four view panes for monitoring different aspects of the run.



To customize the view panes **Run data**, **Curves**, **Flow scheme** and **Logbook**, right-click in the respective view pane and select **Properties**. An alternative is to select **View:Properties**.

For more information about customizing the view panes, see the UNICORN user manuals.

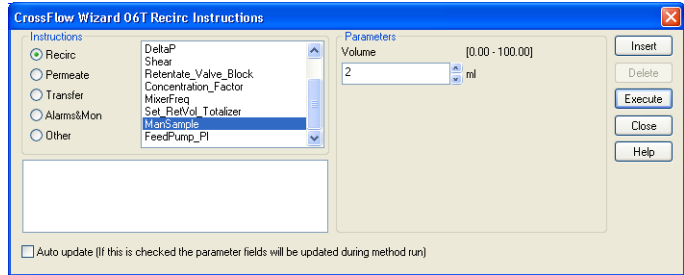
To stop the run before it is finished, click the **End** button above the **Run data** view pane.

Note: If the run is in Hold and the run pauses, it is necessary to click **Hold** to continue.

9.5 Manual sampling during run

It is possible to perform sampling from the reservoir during a run and compensate for the sample volume.

- 1 In **UNICORN**, select **Manual:Recirc** and select the instruction **ManSample**.



- 2 Open the lid of the reservoir,
- 3 Enter the planned sample volume.
- 4 Take a sample with an appropriate pipette device.
- 5 Immediately, click **Execute** in the ManSample dialog.

Note: If not clicked immediately, the system will start to compensate the lost volume before ManSample instruction is used.

- 6 Close the lid.

Note: To get an Eval mark in the result file, it may be convenient to set an Eval_Mark using the instruction **Set_Eval_Mark** with the parameter **ExtData_vs_Capacity**.



10 After run procedures

10.1 System sanitization

If you want to be sure that the system is clean, for example when a new filter is used or a process is changed, it is recommended to perform a sanitization of the system.

CAUTION! Instead of a filter, use a “three way connector” (18-1170-59). Filters are not compatible with the used flows and pressures in the System sanitization method.

Replace the pH electrode with a dummy electrode.

Remove the reservoir float.

If an air filter is connected to the reservoir, remove the filter. Connect the tubing “Reservoir cleaning kit” 11-0033-86 at the reservoir top. Place the tubing in waste.



WARNING! The reservoir is over-filled during the sanitization. It is important to have tubing (11-0033-86) to waste. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective lab coat.

Note: The stirrer should be present in the reservoir during sanitization. However, it should be replaced with a new aseptic one after the sanitization.

Note: The System sanitization method can also be used to pH neutralize the system solutions after sanitization and to exchange the solution in the system to an appropriate storage solution, for example 20% Ethanol.

10.1.1 Sanitization of the pump piston rinsing system

When performing system sanitization, the pump piston rinsing system should be sanitized by using 1 M NaOH as liquid in the rinsing system bottles.

10.1.2 Sanitization of reservoir float

The reservoir float has to be sanitized separately. Remove it from the reservoir before system sanitization. The float can be autoclaved, or equivalent.

10.1.3 Creating a method



- 1 In the **Method Editor** of UNICORN, open the Method Wizard.
- 2 Select the System.
- 3 In the **Basic settings** dialog, select System Sanitization as method.

Basic Settings

Method

- 4 Enter Recirculation time. A minimum recirculation time of 30 min is recommended.

Recirculation Time min (0.999999 min)

- 5 Select the Reservoir used.

System setup

Reservoir Size

☒ 350 ml

☐ 1100 ml

Note: Information on important preparations and notes before the method is run, is displayed in the **Basic Settings** dialog. All In and Outlet connections will be used.

- 6 Click **Finish**.
- 7 In the **Summary** dialog, click **Print** to print the summary. Click **Finish** to end the Method Wizard.

Note: Do not edit any variables or method instructions.

- 8 Save the method.

10.1.4 Running the System sanitization method

When running a method using a sanitization solution containing sodium hydroxide (NaOH):



WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective lab coat.

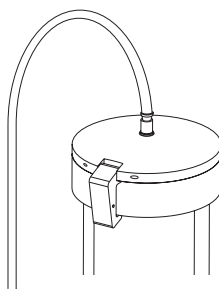
CAUTION! Always make sure that the system components are compatible with sodium hydroxide at the concentration, time and temperature used.

- 1 Prepare a sanitization solution which is compatible with the ÄKTAcrossflow system, for example 1 M NaOH.

Reservoir 350 ml: 5000 ml

Reservoir 1100 ml: 7000 ml

- 2 Fill the Rinsing system bottles with 1 M NaOH (2×200 ml).
- 3 Replace the filter device with a three-way connector.
- 4 Replace the pH electrode with a dummy electrode.
- 5 Remove the float from the reservoir.
- 6 If an air filter is connected to the reservoir, remove the filter. Connect a tubing to waste. The tubing is included in the Reservoir cleaning kit (11-0033-86).



WARNING! The reservoir is over-filled during the sanitization. It is important to have tubing to waste. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective lab coat.

Note: To avoid siphoning back into the reservoir, keep tubing free from solution in the waste bottle.

- 7 Place all inlet tubing in the prepared sanitization solution.
- 8 Place all outlet tubing in a waste container.

-
- 9 Run the method.

Note: *It is also possible to run System sanitization as an Instant Run, see Section 9.3.*

- 10 Empty the system rinsing bottles and fill them with ordinary rinsing solution, 20% ethanol in 10 mM NaOH.
- 11 After the System sanitization method, repeat the method using Milli-Q water or buffer instead of sanitization solution.
- 12 Empty the system rinsing bottles and fill them with rinsing solution, 20% ethanol in 10 mM NaOH.
- 13 Replace the stirrer with a new aseptic one.

10.2 Viewing and printing the result

This section describes the basics of how to view and print the result in the **Evaluation** module.

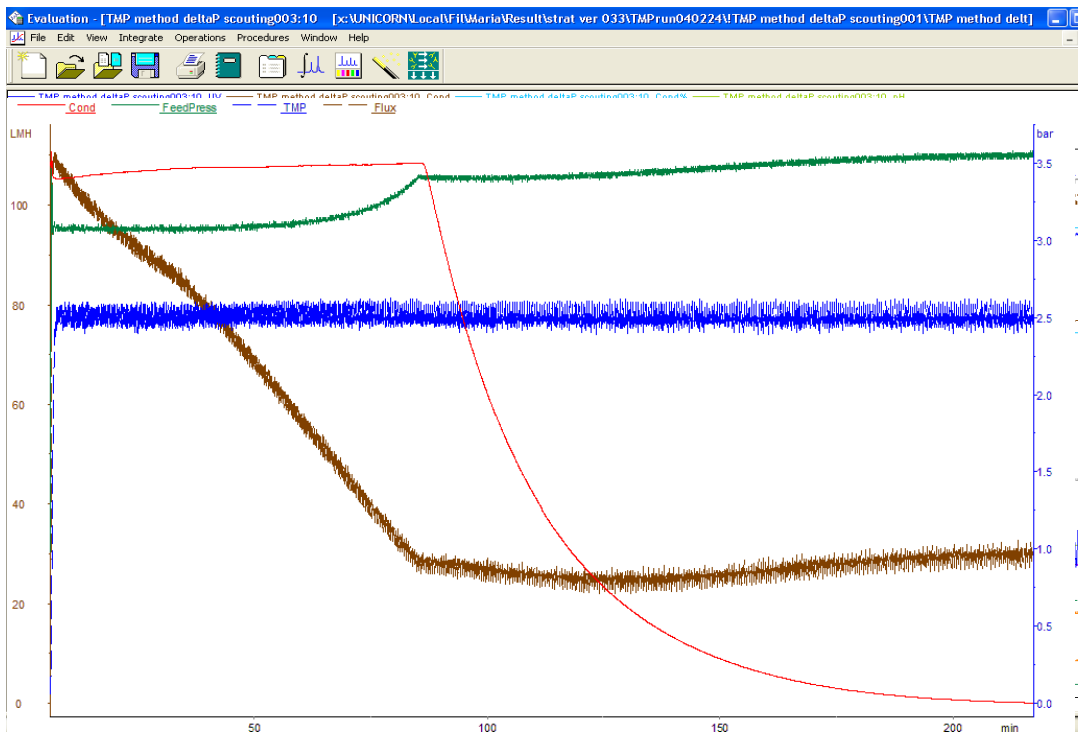
For evaluating ÄKTAcrossflow results, see Chapter 11 and *User Reference Manual - UNICORN 5.1 - Evaluation for Cross Flow Filtration*

10.2.1 Viewing the result

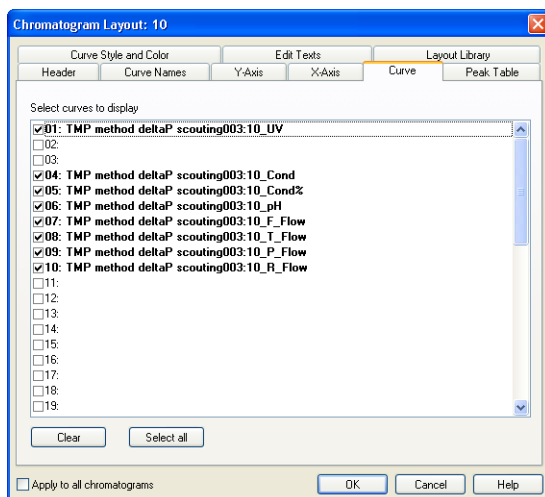
To view the result:

- 1 Locate the result file in the **Results** pane in the **Main Menu** module.
- 2 Double-click the file.

The result file opens in a **Chromatogram** window in the **Evaluation** module.
See below.



- 3 To change the layout of the **Chromatogram** window, right-click in the window and select **Properties**.



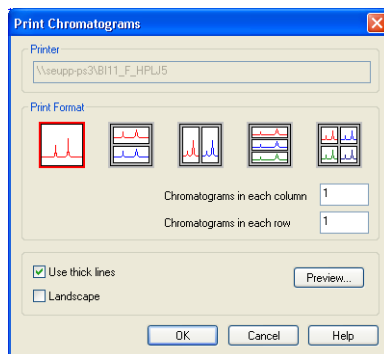
- 4 In the **Curve** tab page, uncheck the check boxes to remove the curves you do not want to display.
- 5 Click **OK**.

Refer to the UNICORN user manuals, Online Help and the *User Reference Manual - UNICORN 5.1 - Evaluation for Cross Flow Filtration* for more information about viewing the result and customizing the layout.

10.2.2 Printing the result

To print the chromatograms:

- 1 Open all chromatograms you want to print in the **Evaluation** module.
- 2 Select the **File:Print** command.
- 3 In the **Print Chromatograms** dialog, select print formats and layout options.



- 4 Click **Preview**.
- 5 In the **Customise Report** window, verify that the layout is correct.
- 6 Click **Edit Mode** to make changes. Click **Preview** to return to the preview mode.
- 7 Click **Exit** to return to the **Print Chromatograms** dialog.
- 8 Click **OK**.

Refer to the UNICORN user manuals for more information about printing results.



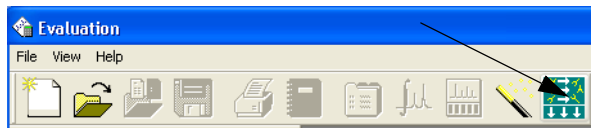
11 Evaluating ÄKTAcrossflow results

11.1 Associated documentation

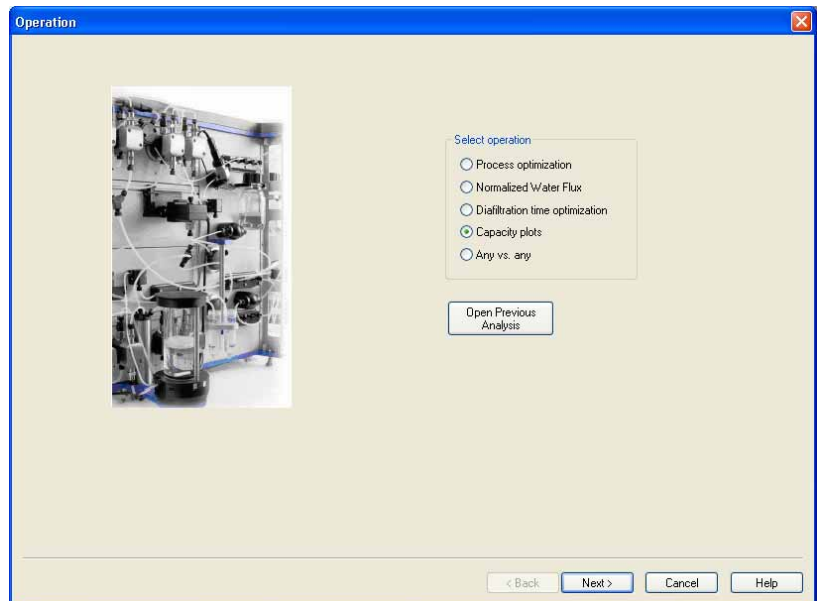
The use of the **Crossflow Evaluation** module in UNICORN is described in detail in the *User Reference Manual - UNICORN 5.1 - Evaluation for Cross Flow Filtration*.

11.2 Opening the Evaluation module

In UNICORN Evaluation click the Membrane System Evaluation icon.



Operation dialog is displayed.



11.3 Main functions

11.3.1 Any vs any

Used to generate a plot of any combination of process data collected during a ÄKTAcrossflow run. The process data could be for example detector signals, flows, flux, and pressures.

11.3.2 Process optimization

Used to get a graphical analysis of process optimization run(s). For example a process optimization to find an optimal flux as a function of TMP.

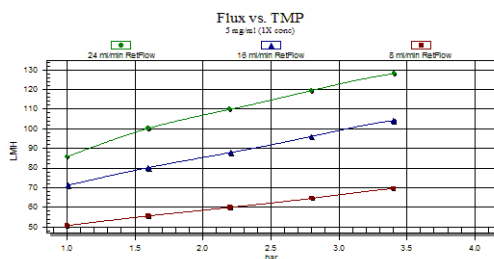
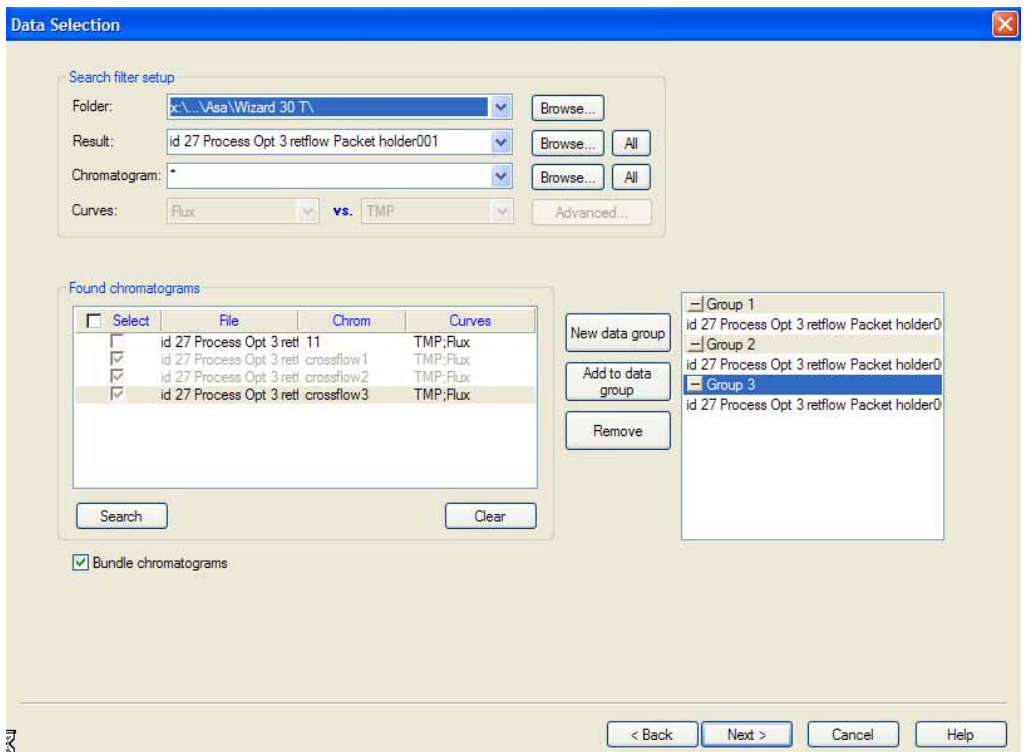


Fig 11-1. Example of process optimization.

Feature in the Evaluation module

The example is a result from a run with up to three different cross flows in the same run.

- 1 When a process result has been selected, "Found chromatograms" are displayed.
- 2 Check the box **"Bundle chromatograms"** and chromatogram groups will be displayed to the right.
- 3 Check the box for the first chromatogram, e.g. "crossflow 1"
- 4 Click the **New data group** button.
- 5 Check the box for the second chromatogram, "crossflow 2" and click **New data group**.
- 6 Repeat the procedure until all chromatograms have been selected as groups.
- 7 Click **Next** to continue the evaluation.



11.3.3 Normalized Water flux

Is used to get normalized water flux values calculated. The results can be used to maintain long term analysis of the filter recovery.

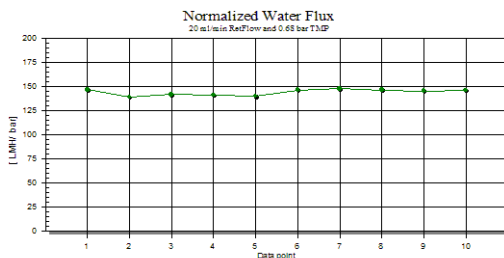


Fig 11-2. Example of plotted Normalized Water Flux.

11.3.4 Capacity plots

Is used to get a graphical analysis of the filter capacity during a filtration.

12 Feedback tuning and PID parameters

12.1 PID control

Whenever an automated process step requires the control of pressure (feed, retentate and permeate pressure), the UNICORN control software of ÄKTAcrossflow employs PID-type controllers to control the pressure to its setpoint. Recommended default settings for the controllers (P, PI or PID type) are listed in Table 12-1. These settings provide fast response and robust control for most operating situations. However, the following controllers may require adjustment depending on the type and behaviour of the filter:

12.1.1 Feed pump control (FeedPressure_PI, DeltaP_PI and EmptyResFeed_PI)

These three controllers ramp the flow rate of the feed pump to achieve a desired pressure. The default settings are appropriate for Flat Sheet Cassettes that give higher back pressure than Hollow Fiber cartridges at a given flow rate. When using Hollow Fiber cartridges, the action of the Integral controller (I parameter) is recommended to be increased (by reducing the figure for the I parameter) to yield a faster pump response and shorter ramp time, respectively. For the alternative settings, see Table 12-1.

When creating methods with the Method Wizard, the alternative settings in Table 12-1 are automatically installed for the different process steps when appropriate.

12.1.2 TMP control (TMP_PID_PermeatePump, TMP_PID_RetentateControlValve)

During TMP control, the permeate pump controls the permeate pressure by adjusting the flow rate of the permeate pump. Depending on membrane area, filter cut-off and process conditions, the magnitude of the permeate flow rate may vary in a wide range from approx. 1 ml/min to 50 ml/min and higher. Two settings are recommended to provide fast and robust control, see also Table 12-1.

- PID settings for process steps where the membrane is not exposed to proteins, thus avoiding the risk for membrane fouling or gel-layer formation. For these steps, a high permeate flow rate and a rather linear pressure-flow behaviour of the filter is typical.

- PID settings for process steps with risk of membrane fouling or gel-layer formation, like product steps. Under these conditions, a slower but more robust control accounts for low permeate flow rates and a non-linear pressure-flow behaviour. These “slow” settings are selected as default values in the strategy to provide maximum robustness of the control.

Control element	PID settings	P	I	D
Feed pump control	Hollow Fibers:			
	FeedPressure_PI	0.05	50	NA
	DeltaP_PI	0.05	20	NA
	EmptyResFeed_PI	0.05	20	NA
	Flat Sheet Cassettes:			
	FeedPressure_PI	0.05	150	NA
	DeltaP_PI	0.05	150	NA
	EmptyResFeed_PI	0.05	150	NA
TMP control	TMP_PID_RetentateControlValve	0.1	20	1
	TMP_PID_PermeatePump (<i>membrane not exposed to proteins</i>)	0.1	20	0
	TMP_PID_PermeatePump (<i>membrane exposed to proteins</i>)	0.03	300	0
	TMP_PID_PermeatePump (hollow fibers)	0.05	75	
PUF control	PUF_PI_RetentateControlValve	0.1	20	NA
	PUF_PI_PermeatePump	0.001	200	NA
Flux control	Flux_PI_RetentateControlValve	0.1	20	NA
NFF control	pNFF_PI	0.2	20	NA
		0.05	150	
Level control	Const_RVol_P	50	NA	NA

Table 12-1. Recommended settings for PID control (strategy default settings in bold style)

12.1.3 Tuning and troubleshooting of PID control

If tuning of the PID control is required, it is most likely that control settings for feed pump or permeate pump need to be adjusted. The settings for the Retentate Control Valve given in Table 12-1 should not be modified at all. Common methods for PID optimization (e.g. Ziegler-Nichols method) can be applied.

In most situations, good results are obtained when using the following rules of thumb:

- 1 Slower control: Increase I parameter, decrease P parameter.
Faster control: Decrease I parameter, increase P parameter.
- 2 No drastic changes should be applied, an appropriate measure is to change the I parameter by factor $\frac{1}{2}$ or 2, respectively, while initially keeping the P parameter constant.

12.1.4 PI parameters for larger filter areas

When using larger filter areas, e.g. 100 cm^2 , it is necessary to optimize the parameters.

When optimizing the parameters to obtain a faster regulation, a recommended start may be to:

- Decrease I parameter by dividing with 2.
- Increase P parameter by multiplying with 1.2
- An example for the parameters of TMP_PID_RetentateControlValve is to:
 - Decrease I parameter from 20 to 10
 - Increase P parameter from 0.1 to 0.12

When the regulation is too fast, e.g. noisy curves, it may be necessary to optimize the parameters in the opposite way.

- Increase I parameter and decrease P parameter

12.2 *Hardware components using PI and PID parameters.*

PI and PID parameters are used for the following Hardware components and Instructions. See Table 12-2 .

Hardware component	Instruction
Feed Pump	Feedpump_Pi
	DeltaP_Pi
Retentate Control Valve	TMP_PID_RetentateControlValve
	Flux_Pi_RetentateControlValve
	PUF_Pi_RetentateControlValve
Permeate Pump	TMP_PID_PermeatePump
	PUF_Pi_PermeatePump
	pNFF_Pi
Reservoir	ConstRVol_P
	EmptyResFeed_Pi

Table 12-2. Hardware components using PI parameters.

12.3 Description of the PI parameters and regulators

The table below describes the three PID parameters used.

Parameter	Description
P	The P parameter reduces the effect of an error but does not completely eliminate it. A simple P-regulator results in a stable stationary error between actual and requested flow or pressure.
I	The I parameter eliminates the stationary error, but results in a slight instability leading to oscillations in the actual flow or pressure. The I parameter can have values between 0 and infinity. Smaller values have a greater effect and a value of infinity has no effect. <i>Note:</i> The value infinity is set as 9999 in UNICORN.
D	In certain cases, the D parameter can reduce the oscillations introduced by a PI-regulator. D can have values between 0 and infinity, where larger values have a greater effect and a value of 0 has no effect. <i>Note:</i> Most often, a simple PI-regulator is preferable for control of pressure, and ÅKTAcrossflow is therefore configured by default with the D parameter set to zero.

Table 12-3. PID parameters.

12.4 Setting up feedback tuning

UNICORN uses so-called PI feedback tuning, where P and I are parameters that determine the tuning characteristics.

There are two ways to apply the feedback tuning instructions:

- In the **Method Editor** module.
- In the **System Control** module, reached with the commands **Manual** or **System Settings**.

12.4.1 Instruction groups

The instructions are found in the following Instruction groups, see Table 12-4 .

Instruction name	Instruction Group
FeedPressure_PI	System Settings:Specials Method/Manual: Recirculation
TMP_PID_RetentateControlValve	System Settings: Specials Method/Manual: Permeate
TMP_PID_PermeatePump	System Settings:Specials Method/Manual: Permeate
Flux_PI_RetentateControlValve	System Settings: Specials Method/Manual: Permeate
PUF_PI_RetentateControlValve	System Settings: Specials Method/Manual: Permeate
PUF_PI_PermeatePump	System Settings:Specials Method/Manual: Permeate
pNFF_PI	System Settings:Specials Method/Manual: Permeate
DeltaP_PI	System Settings:Specials Method/Manual: Recirculation
ConstRVol_P	System Settings:Specials Method/Manual: Transfer
EmptyResFeed_PI	System Settings:Specials Method/Manual: Recirculation

Table 12-4. PI instruction groups.

12.4.2 Description of the PID instructions.

In Table 12-5 the PID instructions are described.

Instruction name	Description
FeedPressure_PI	These parameters are used to tune the feedback control when Feed_Pressure_Control is active.
TMP_PID_RetentateControlValve	These parameters are used to tune the feedback control when TMP_Control is active.
TMP_PID_PermeatePump	These parameters are used to tune the feedback control when TMP_Control is active.
Flux_PI_RetentateControlValve	These parameters are used to tune the feedback control when Flux_Control is active.
PUF_PI_RetentateControlValve	These parameters are used to tune the feedback control when Permeate_Unrestricted_Flow is active.
PUF_PI_PermeatePump	These parameters are used to tune the feedback control when Permeate_Unrestricted_Flow is active.
pNFF_PI	These parameters are used to tune the feedback control when Normal_Flow_Filtration is active.
DeltaP_PI	These parameters are used to tune the feedback control when DeltaP is active.
ConstRVol_P	This parameter affects the responsiveness of the CRV algorithm. It controls how fast the transfer pump will compensate changes of the reservoir volume when using ConstantRetentateVolume.
EmptyResFeed_PI	This instruction allows manipulation of the P and I parameters of the regulator algorithm used to keep the feed pressure at the requested level during the EmptyReservoir procedure.

Table 12-5. Descriptions of PI instructions.

12.5 Optimizing the PI parameters

The objective to optimize the PI parameters is to obtain a:

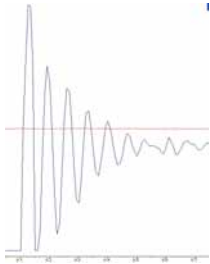
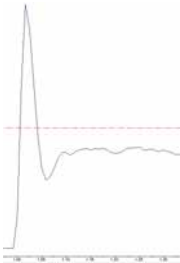
- Smooth regulation,
- without over-pressure peaks, but
- fast enough.

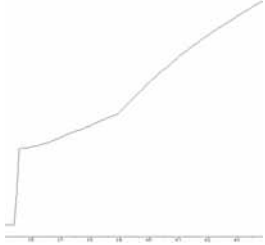
Too weak PI values may lead to a smooth, but slow ramping.

Too aggressive PI values may lead to a fast, but instable ramping.

12.5.1 Regulation of the Feed pump, example.

The Feedpump_PI instruction was used when the system was run manually. The Feed pump was run using different PI parameter values to obtain a set Feed pressure

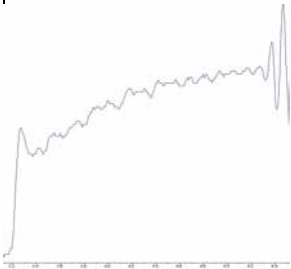
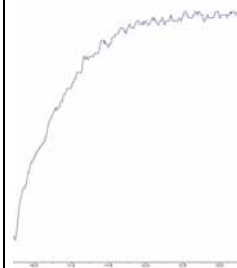
PI settings	Curve example (Feed pump flow)
1) Settings: The I parameter was set to a high value (1000) which means it has minimal effect of the regulation. The P parameter was set to a high value (1.0). Result: Totally uncontrolled regulation with high fluctuations.	
2) Settings: The I parameter was kept to 1000. The P parameter was set to 0.5. Result: The size of the first step is too large.	

PI settings	Curve example (Feed pump flow)
3) Settings: The I parameter was kept. The P parameter was set to 0.2. Result: The size of the first step is still too large.	
4) Settings: The I parameter was kept. The P parameter was set to 0.1. Result: The size of the first step is still too large.	
4b) Settings: The I parameter was kept. The P parameter was set to 0.05. Result: The size of the step seems to be correct.	
5) Settings: The P parameter is kept to 0.05. The optimization of the I parameter starts with the settings 500 and continues with I=200. Result: The slope of the curve is increasing with decreased I values.	

PI settings	Curve example (Feed pump flow)
6) Settings: The I parameter is further decreased (200,100). Result: Increased slope is obtained.	
7) Settings: The P parameter was kept at 0.05 and the I parameter was set to 25. Result: The curve indicates a too high flow before it stabilizes. The I parameter should be increased.	
9) Settings: The I parameter is increased to 50. Result: The PI parameters are optimized and a smooth and fast regulation is obtained.	

12.5.2 Regulation of the Permeate pump, example.

The TMP_PI_PermeatePump instruction was used when the system was run manually. TMP Control was set to 1 Bar and was monitored at different PI values in the instruction.

PI settings	Curve example (TMP)
1) Settings: The P parameter was set to 1. The I parameter was set to 100. Result: Pressure peaks were observed both in the beginning of the regulation and also when TMP was reached.	 A line graph showing the response of a pressure control system. The y-axis represents pressure, and the x-axis represents time. The curve starts at a baseline, rises sharply with a significant overshoot, and then exhibits damped oscillations before settling at a steady-state value. The x-axis has numerical markings from 0 to 25.
2) Settings: The P parameter was decreased to 0.2. The I parameter was decreased to 50. Result: Smooth regulation up to set TMP and no pressure peaks were observed when TMP was reached.	 A line graph showing the response of a pressure control system. The y-axis represents pressure, and the x-axis represents time. The curve starts at a baseline, rises smoothly and monotonically, and then levels off at a steady-state value without any overshoot or oscillations. The x-axis has numerical markings from 0 to 25.



13 Strategy instructions

13.1 System settings and instruction boxes

13.1.1 System setting: The order of instructions within each group.

Alarms/ Warnings	Specials	Monitors	Curves
UV	RetentateHoldUpVol	AveragingTimeUV	UV
pH	PressureOffset	Pressure_Filter_Factor	Cond
Cond	RPCVoffset	CondTempComp	FeedFlow
Feed_Press	RPCVhysteresis	CondRefTemp	RetFlow
Trf_Press	PPCV_Setp	pHTempComp	PermFlow
DeltaP	Total_Membrane_Area	AirSensor	TrfFlow
TMP	Lumen_Diameter	WatchPar_UV	Feed_Press
Flux	Total_Number_of_Fibers	WatchPar_pH	RetenPress
Shear		WatchPar_Cond	PermPress
pNFF	AuxOut1	WatchPar_Feed_Press	pH
Valves	AuxOut2	WatchPar_Reten_Press	Temp
Flowpath	AuxOut3	WatchPar_PermPress	DeltaP
AirSensor	AuxOut4	WatchPar_FeedFlow	TMP
ZeroLevel	FeedPressure_Pi	WatchPar_Ret_Flow	Flux
	DeltaP_Pi	WatchPar_PermFlow	Shear
	TMP_PID_RetentateControlValve	WatchPar_Trfflow	ConcFact
	TMP_PID_PermeatePump	WatchPar_RetVol	DF_X_Fct
	Flux_Pi_RetentateControlValve	WatchPar_ResVol	RetVol
	PUF_Pi_RetentateControlValve	WatchPar_PermVol	ResVol
	PUF_Pi_PermeatePump	WatchPar_TransVol	PermVol
	pNFF_Pi	WatchPar_DeltaP	TransVol
	EmptyResFeed_Pi	WatchPar_TMP	ConvRatio
	ConstrVol_P	WatchPar_Flux	pNFF
	FracParameters*	WatchPar_Shear	AuxIn1
	Frac_Numbering_Mode*	WatchPar_pNFF	AuxOut1
	Reservoir_Size	WatchPar_ConcFact	
		WatchPar_DF_X_Fact	
		WatchPar_%Flux_Drop	

*Some instructions are only shown when chosen as components

13.1.2 Instruction box: The order of instructions within each group.

Note: Only groups defined by the strategy are described.

Recirc	Permeate	Transfer	Alarms, Warnings and Monitors	Watch ¹
FeedFlow	TMP_Control ²	Trans_Flow	AutoZeroUV	Hold_Until
RetFlow ²	Flux_Control ²	Constant_Ret_Volume ²	AveragingTimeUV	Watch_UV
FeedPressure ²	Permeate_Valve_Block ²	Transfer_Valve_Blocks	Pressure_Filter_Factor	Watch_pH
DeltaP ²	Permeate_Unrestricted_Flow ²	Transfer_Purge_Valve	Alarm_UV	Watch_Cond
Shear ²	NFF_ConstantFlow	Set_TrFVol_Totalizer	Alarm_pH	Watch_FeedPress
Retentate_Valve_Block	NFF_ConstantPressure	DF_ExchangeFactor ²	Alarm_Cond	Watch_RetenPress
EmptyReservoir	Start_Eval_Window ²	Method_Base ¹	Alarm_FeedPress	Watch_PermPress
EmptyReservoirAbort	Stop_Eval_Window ²	ConstRVol_P	Alarm_TrFPress	Watch_FeedFlow
Concentration_Factor ²	Set_Eval_Mark ²		Alarm_DeltaP	Watch_Reten_Flow
MixerSpeed	Set_PermVol_Totalizer		Alarm_TMP	Watch_PermFlow
Set_ResVol_Totalizer	%Flux_Drop_Calc ²		Alarm_Flux	Watch_TrFFlow
ManSample ⁴	Total_Membrane_Surface_Area		Alarm_pNFF	Watch_RetVol
FeedPressure_PI	Lumen_Diameter ^{v2}		Alarm_Shear	Watch_ResVol
DeltaP_PI	Total_Number_Of_Fibers ²		Alarm_Valves	Watch_PermVol
EmptyResFeed_PI	TMP_PI_RetentatControlValve ²		Alarm_Flowpath	Watch_TransVol
RetentateHoldupVol	TMP_PI_PermeatePump ²		Alarm_AirSensor	Watch_DeltaP
	Flux_PI_RetentatControlValve ²		Alarm_ZeroLevel	Watch_TMP
	PUF_PI_RetentatControlValve ²		WatchPar_UV	Watch_Flux
	PUF_PI_PermeatePump ²		WatchPar_pH	Watch_Shear
	pNFF_PI		WatchPar_Cond	Watch_pNFF
	Fractionation ^{2, 5}		WatchPar_FeedPress	Watch_ConcFact
	FractionationStop ⁵		WatchPar_RetenPress	Watch_DF_X_Fact
	ResetTubeNumber		WatchPar_PermPress	Watch_Flux_Drop
	FeedTube ⁵		WatchPar_FeedFlow	Watch_AirSensor
			WatchPar_Reten_Flow	Watch_ZeroLevel
			WatchPar_PermFlow	Watch_AuxIn1
			WatchPar_TrFFlow	Watch_AuxIn2
			WatchPar_ResVol	Watch_AuxIn3
			WatchPar_RetVol	Watch_AuxIn4
			WatchPar_PermVol	Watch_Off
			WatchPar_TransVol	
			WatchPar_DeltaP	
			WatchPar_TMP	
			WatchPar_Flux	
			WatchPar_Shear	
			WatchPar_pNFF	
			WatchPar_ConcFactortor	
			WatchPar_DF_X_Fct	
			WatchPar_%Flux_Drop	
			UVlampOFF	
			LevelSensorCalibration	

1)*Only in Method Editor. 2)*Only in Method Editor and System control when running a method.

3)*Only in System Control when not running a method. 4)*Only in System control.

5)*Note! Some instructions are only shown when chosen as components, e.g. Frac. 6)* Only for Hollow fibers.

13.2 Recirculation instructions

13.2.1 FeedFlow

Instruction name FeedFlow	Formula: Qf	Group Method/Manual: Recirc
Parameter 1 name FlowRate	Mode Control Mode Direct	Position name (default underlined) <u>0.0</u> ml/min (0-600)
Instruction Help text <i>Starts the flow on the feed pump. Feed Flow should stabilize at 98 % of set value before a new instruction is executed. To allow new instruction after stabilized DeltaP, "Watch_Stable_Baseline DeltaP" should be programmed in methods. Instruction resets RetFlow , FeedPressure DeltaP and Shear.</i>		

13.2.2 RetFlow

Instruction name RetFlow	Formula: Qr=Qf-Qp	Group Method/Manual: Recirc
Parameter 1 name FlowRate	Mode Control Mode Direct	Position name (default underlined) <u>0.0</u> ml/min (0.0-600.0)
Instruction Help text <i>Starts the flow on the feed pump. RetFlow should stabilize at 98% of set value before new instruction is executed. Instruction resets FeedFlow, FeedPressure, DeltaP and Shear. To allow new instruction after stabilized DeltaP, "Watch_Stable_Baseline DeltaP" should be programmed in methods.</i>		

13.2.3 FeedPressure

Instruction name FeedPressure	Formula: Qp	Group Method/Manual: Recirc
Parameter 1 name Pressure	Mode Control Mode Direct	Position name (default underlined) <u>0.00</u> bar (0.00-5.20)
Instruction Help text <i>Starts the flow on the feed pump. Feed Pressure should stabilize at 98% of set value before new instruction is executed. Instruction resets FeedFlow, RetFlow , DeltaP and Shear. To allow new instruction after stabilized DeltaP "Watch_Stable_Baseline DeltaP" should be programmed in methods</i>		

13.2.4 DeltaP

Instruction name DeltaP	Formula: DeltaP=Pf-Pr	Group Method/Manual: Recirc
Parameter 1 name Pressure	Mode Control Mode PID	Position name (default underlined) <u>0.0</u> bar (0-5.2)
Instruction Help text <i>Starts the flow on the feed pump. DeltaP should stabilize at 98% of set value before new instruction is executed. Instruction resets FeedFlow, Shear and FeedPressure.</i> <i>To allow new instruction after stabilized DeltaP "Watch_Stable_Baseline DeltaP" should be programmed in method</i>		

13.2.5 Shear

Instruction name Shear	Formula: Shear=(4*Qf) / (No. of Fibers*π*(fiberradius)³)		Group Method/Manual: Recirc
Parameter 1 name Shear	Mode Control Mode Direct	Note Only for HF	Position name (default underlined) <u>0</u> s ⁻¹ (0-20000)
Instruction Help text <i>Starts the flow on the feed pump. Shear should stabilize at 98% of set value before new instruction is executed. Instruction resets FeedFlow, FeedPressure, DeltaP and RetFlow.</i> <i>To allow new instruction after stabilized Shear, "Watch_Stable_Baseline DeltaP" should be programmed in method.</i>			

13.2.6 Retentate_Valve_Block

Instruction name Retentate_Valve_Block	Group Method/Manual:Recirc
Parameter name Macro	Position name (default underlined) <u>R-VB-Recycle</u> , R-VB-Out1, R-VB-Out2, R-VB-Out3
Description Selects the position for R-VB-Recycle, R-VB-Out1(Port located prior to filter devise), R-VB-Out2, R-VB-Out3 When Feed_flow > 0 or Constant_Retentate_Volume is active no action is allowed. A warning will be raised.	
Instruction Help text <i>Selects the position for R-VB-Recycle, R-VB-Out1(Port located prior to filter devise), R-VB-Out2, R-VB-Out3</i> <i>When Feed_Flow > 0 or Constant_Retentate_Volume is active no action is allowed. A warning will be raised.</i> <i>Alarm help text 1: Retentate_Valve_Block instruction not allowed during Constant_Retentate_Volume</i> <i>Alarm help text 2: Retentate_Valve_Block instruction not allowed when Feed setpoint > 0</i> <i>Alarm help text 1: Retentate_Valve_Block instruction not allowed during EmptyReservoir</i> <i>Alarm help text 2: Retentate_Valve_Block instruction not allowed when during NFF</i>	

13.2.7 RPCVoffset

Instruction name RPCVoffset	Group System:Settings: Specials
Parameter name Pressure	Set point (default underlined) <u>350</u> , (0 – 999)
Instruction Help text <i>Sets offset on RetentateControlValve used by system during TMP_Control, Flux_Control and Permeate_Unrestricted_Flow</i>	

13.2.8 RPCVhysteresis

Instruction name RPCVhysteresis	Group System:Settings: Specials
Parameter name Setpoint	Set point (default underlined) <u>50</u> , (0 – 100)
Description	
Instruction Help text	

13.2.9 Reservoir_Size

Instruction name Reservoir_Size	Group System:Settings: Specials,
Parameter 1 name Size	Position name (default underlined) <u>350 ml</u> , (350 , 1100 ml)
Instruction Help text <i>Sets reservoir size 350 or 1100 ml</i>	

13.2.10 EmptyReservoir

Instruction name EmptyReservoir	Group Method/Manual: Recirc
Parameter 1 name RetValveOutlet	Position name (default underlined) <u>R-VB-Out1</u> , R-VB-Out2, R-VB-Out3
Parameter 2 name MaxFeedPressure	Position name (default underlined) <u>0.00 bar</u> (0.00 – 5.20 bar)
Parameter 2 name MaxFeedFlow	Position name (default underlined) <u>600.0 ml/min</u> , (0.0 – 600.0 ml/min)
Instruction Help text <i>Instruction uses R-VB-Out to open one position, FeedPump to empty reservoir in a controlled manner. In order to perform this, FeedFlow is reduced when reservoir level is below a certain value and R-VB-Out creates a back pressure to avoid siphoning, about 2 bar. When 0-level is reached, defined as just below reservoir bottom surface, Feedflow is immediately stopped.</i>	

13.2.11 EmptyReservoirAbort

Instruction name EmptyReservoirAbort	Group Method/Manual: Recirc
Parameter 1 name RetValvePort	Position name (default underlined) <u>R-VB-Recycle</u> ,
Parameter 2 name FeedFlow	Position name (default underlined) <u>0.0 ml/min</u> , (0.0 – 600.0 ml/min)
Instruction Help text <i>EmptyReservoirAbort aborts the sequence initiated by the EmptyReservoir instruction. Instruction immediately stops Feedflow. Sets R-VB-Out to Recycle position. Eventually, Watch EmptyResFinished signal is generated.</i>	

13.2.12 Concentration_Factor

Instruction name Concentration_Factor	Group Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) <u>Off_On</u>
Parameter 2 name Type	Position name (default underlined) <u>FedBatch</u> _TankBatch
Instruction Help text <i>Starts the Concentration factor calculation Use watch command in method to set the endpoint. FedBatch cannot be operated without a Set_ResVol_Totalizer volume entered. Detailed information in User Manual.</i>	

13.2.13 MixerSpeed

Instruction name MixerSpeed	Group System:Settings: Specials, Manual, Method
Parameter 1 name Speed	Position name (default underlined) <u>Auto</u> , (0 - 600 rpm)
Instruction Help text <i>Sets Mixer speed, Auto adjusts mixer speed to reservoir size (SystemSettings) and actual ResVol in a linear relation. Min-Speed 80 rpm Max Speed for 350ml 200 rpm and Max Speed for 1100ml 300 rpm</i>	

13.2.14 Set_ResVol_Totalizer

Instruction name Set_ResVol_Totalizer	Group Method/Manual: Recirc
Parameter name Volume	Set point (default underlined) <u>0.0</u> ml (0.00 – 5000.00)
Instruction Help text <i>Sets a desired volume for totalizer. Transfer pump flow will increase value, if it is directed to reservoir. Permeate flow, if permeate outlet valve is opened will decrease volume, but not during position Recycle of the Permeate_Valve_Block and if Transfer_Purge_Valve is in position reservoir. Feed flow with retentate valve open will also decrease volume.</i>	

13.2.15 ManSample

Instruction name ManSample	Group Manual: Recirc
Parameter 1 name Volume	Position name (default underlined) <u>0.00</u> ml (0.00-100.00)
Instruction Help text <i>Reduce totalizer manually with the amount of removed sample at that very moment. See equation under Concentration_Factor in User Manual. It is very important that a correct value is inserted ASAP.</i>	

13.2.16 FeedPressure_PI

Instruction name FeedPressure_PI	Group System Settings:Specials, Method/Manual: Recirc
Parameter 1 name P	Position name (default underlined) <u>0.050</u> (0.000-10000.000)
Parameter 2 name I	Position name (default underlined) <u>150.000</u> sec (0. 000 -10000.000)
Instruction Help text <i>These parameters are used to tune the feedback control when FeedPressurecontrol is active. For details, see "Feedback tuning" chapter in User/Instrument Manual .</i>	

13.2.17 DeltaP_PI

Instruction name DeltaP_PI	Group System Settings:Specials, Method/Manual: Recirc
Parameter 1 name P	Position name (default underlined) <u>0.100</u> (0.000-10000.000)
Parameter 2 name I	Position name (default underlined) <u>10.000</u> sec (0. 000-10000.000)
Instruction Help text <i>These parameters are only used to tune the feedback control during DeltaP. For details, see "Feedback tuning" chapter in User/Instrument Manual .</i>	

13.2.18 EmptyResFeed_PI

Instruction name EmptyResFeed_PI	Group System Settings:Specials, Method/Manual: Recirc
Parameter 1 name FPC_P	Position name (default underlined) <u>0.05</u> (0.000 -10000.000)
Parameter 2 name FPC_I	Position name (default underlined) <u>150.000</u> sec (0. 00-10000.000)
Instruction Help text <i>These parameters are only used to tune the feedback control during EmptyReservoir. For details, see "Feedback tuning" chapter in User/Instrument Manual .</i>	

13.2.19 RetentateHoldupVol

Instruction name RetentateHoldupVol	Group System Settings:Specials, Method/Manual: Recirc
Parameter 1 name Volume	Position name (default underlined) <u>18.20 ml</u> (0.00-100.000)
Instruction Help text <i>Sets the calculated or measured value for Holdup Volume</i>	

13.3 Permeate instructions

13.3.1 TMP_Control

Instruction name TMP_Control	Formula: $TMP = ((Pf + Pr) / 2) - Pp$		Group Method/Manual: Permeate
Parameter name Pressure	Mode Control_Mode PID	Note No overshooting (max 10% of set-point)	Position name (default underlined) <u>0.00</u> bar (0.0-5.20 bar)
Instruction Help text <i>Adjusts Retentate_Control_Valve and permeate pump to maintain set TMP. Prior to activation of TMP control make sure that DeltaP is stable. Resets, Flux_Control and Permeate_Unrestricted_Flow</i>			

13.3.2 Flux_Control

Instruction name Flux_Control	Formula: $\text{Flux} = (Q_p[l/h]) / (A[m^2])$		Group Method/Manual: Permeate
Parameter name1 Flux	Mode Control Mode Direct	Note $P_p \leq P_r$	Position name (default underlined) <u>0.0</u> LMH (0.0-4800.0)
Parameter name2			Position name (default underlined)
TMPLimit			<u>Off</u> , (0.01 – 5.20 bar)
Instruction Help text <i>Starts the flow on the permeate pump as flux. If permeate pressure < 0.2 bar R-PCV will lift permeate pressure to Offset. If system reaches TMPLimit when active, system will Pause. Resets TMP_Control, and Permeate_Unrestricted_Flow. RPCV will lift permeate pressure to 95% of Offset before Flux will be ramped up during 30 sec..</i>			

13.3.3 Permeate_Valve_Blocks

Instruction name Permeate_Valve_Block	Group Method/Manual:Permeate
Parameter name Macro	Position name (default underlined) <u>Closed</u> , P-VB-Recycle, P-VB-Out1, P-VB-Out2, P-VB-Out3,
Instruction Help text <i>Selects the position for the Permeate_Valve_Block to P-VB-Recycle, P-VB-Out1, P-VB-Out2, P-VB-Out3. No valve block action allowed when P-VB-recycle is used and a permeate control mode is active. A warning will be raised. During Constant_Retentate_Volume, P-VB-recycle position is not allowed. A warning will be raised. It is possible to change position when permeate flow is greater than 0, although volume will not be accurate. When opening a new valve outlet the old outlet will be open for about one second. Permeate_Valve_Block shall remain in position at Pause and the flow on the pump(s) goes down to 0 ml/min.</i>	
Warning help text 1: <i>Instruction ignored. No valve block action allowed when P-VB-recycle is used and permeate control mode is active.</i>	
Warning help text 2: <i>Instruction ignored. P-VB-recycle is not allowed during Constant retentate volume.</i>	

13.3.4 Permeate_Unrestricted_Flow

Instruction name Permeate_Unrestricted_Flow	Formula: Pp		Group Method/Manual: Permeate
Parameter name Pressure	Mode Control Mode PID	Note Pp ≤Pr	Position name (default underlined) <u>Disabled</u> , Enabled
Instruction Help text <i>Starts the flow on the permeate pump at Offset permeate pressure. If RetenPress > PermPress, RetenPress value is used as Offset. RetentateControlValve lifts RetenPress to Offset if RetenPress < Offset. Resets TMP_Control and Flux_Control.</i>			

13.3.5 Normal_Flow_Filtration

NFF_ConstantFlow

Instruction name NFF_ConstantFlow	Formula: Qf	Group Method/Manual: Permeate
Parameter name Flow	Mode Control Mode Direct	Position name (default underlined) <u>Off</u> , 0.0 ml/min (0-600)
Instruction Help text <i>Starts set feed flow and starts permeate pump at 20% of set feed flow. Retentate-PressureControlValve close retentate loop. Permeate-PressureControlValve is open. Instruction ignored if feed flow >0 ml/min</i>		

NFF_ConstantPressure

Instruction name NFF_ConstantPressure	Formula: Pp		Group Method/Manual: Permeate
Parameter name Pressure	Mode Control Mode PID	Note Pp ≤Pr	Position name (default underlined) <u>Off</u> , 0,0 bar (0.00 – 5.20 bar)
Instruction Help text <i>Starts the flow on the permeate pump at Offset permeate pressure. If RetenPress > PermPress, RetenPress value is used as Offset. RetentateControlValve lifts RetenPress to Offset if RetenPress < Offset. Resets TMP_Control and Flux_Control.</i>			

13.3.6 PressureOffset

Instruction name PressureOffset	Group System:Settings: Specials
Parameter name Pressure	Set point (default underlined) <u>0.2</u> bar (0.2 – 1.0 bar)
Instruction Help text <i>Sets offset pressure used by system during TMP_Control, Flux_Control and Permeate_Unrestricted_Flow</i>	

13.3.7 Evaluation Instructions

Start_Eval_Window

Instruction name Start_Eval_Window	Group Method/Manual: Permeate
Parameter 1 name Mode	Position name (default underlined) <u>0</u> =Any vs any, 1=Capacity, 2=DFTIMEOpt
Instruction Help text <i>Starts collection of chosen sets of data for evaluation. Several Window instructions can be given during a run. For more information see UNICORN manual Evaluation Appendix</i>	

Stop_Eval_Window

Instruction name Stop_Eval_Window	Group Method/Manual: Permeate
Parameter 1 name Mode	Position name (default underlined) <u>0</u> =Any vs any, 1=Capacity, 2= DFTIMEOpt
Instruction Help text <i>Stops collection of chosen sets of data for evaluation. Several Window instructions can be given during a run. For more information see UNICORN manual Evaluation Appendix</i>	

Set_Eval_Mark

Instruction name Set_Eval_Mark	Group Method/Manual: Permeate
Parameter 1 name Mode	Position name (default underlined) <u>0</u> =ProcessOptimisation, 1= ExtData_vs_Capacity, 2= NormalisedWaterflux
Instruction Help text <i>Sets a mark for data collection. Several Mark instructions can be given during a run. For more information see UNICORN manual Evaluation Appendix</i>	

13.3.8 Set_PermVol_Totalizer

Instruction name Set_PermVol_Totalizer	Group Method/Manual: Permeate
Parameter name Volume	Set point (default underlined) <u>0.00</u> ml (0.00 – 5000.00)
Instruction Help text <i>Sets a desired volume for totalizer. Permeate pump flow will increase value.</i>	

13.3.9 %Flux_Drop_Calculation

Instruction name %Flux_Drop_Calculation	Group Method/Manual: Permeate
Parameter 1 name Mode	Position name (default underlined) <u>Off</u> , On
Instruction Help text <i>Latches reference flux and calculates % drop with current flux. Increase in flux values not relevant</i>	

13.3.10 Total_Membrane_Surface_Area

Instruction name Total_Membrane_Surface_Area	Group Method/Manual: Permeate System Settings: Specials
Parameter 1 name Area	Position name (default underlined) <u>50</u> , cm ² , (1 – 1200 cm ²)
Instruction Help text Used for calculation of flux from the equation: $\text{Flux} = (Q_p[l/h]) / (A[m^2])$	

13.3.11 Lumen_Diameter

Instruction name Lumen_Diameter	Group Method/Manual: Recirc . System Settings: Specials
Parameter 1 name Diameter	Position name (default underlined) <u>1.00 mm</u> , (0.1 – 10.0 mm)
Instruction Help text <i>Together with Total_Number_Of_Lumen Shear can be calculated using $\text{Shear} = (4 * Q_f) / (\text{No. of Fibers} * \pi * (\text{fiberradius})^3)$</i>	

13.3.12 Total_Number_of_Fibres

Instruction name Total_Number_Of_Fibers	Group Method/Manual: Recirc . System Settings: Specials
Parameter 1 name Quantity	Position name (default underlined) <u>1</u> , (1 - 1000)
Instruction Help text <i>Together with Lumen_Diameter Shear can be calculated using $Shear=(4*Qf) / (No. of Fibers*\pi*(fiberradius)^3)$</i>	

13.3.13 PPCV_Setp

Instruction name PPCV_Setp	Group System:Settings: Specials
Parameter name Pressure	Set point (default underlined) <u>700</u> , (0 - 999)
Instruction Help text <i>Sets offset on PermeateControlValve used by system during TMP_Control, Flux_Control and Permeate_Unrestricted_Flow</i>	

13.3.14 TMP_PI_RetentateControlValve

Instruction name TMP_PI_RetentateControlValve	Group System Settings: Specials Method/Manual: Permeate
Parameter 1 name P	Setpoint name (default underlined) <u>0.100</u> (0.000-10000.000)
Parameter 2 name I	Setpoint name (default underlined) <u>20.000</u> _sec (0.000-10000.000)
Parameter 3 name D	Setpoint name (default underlined) <u>1.000</u> _sec (0.000-10000.000)
Instruction Help text <i>These parameters are used to tune the feedback control when TMP_Control is active. For details, see "Feedback tuning" chapter in User/Instrument Manual ..</i>	

13.3.15 TMP_PI_PermeatePump

Instruction name TMP_PI_PermeatePump	Group System Settings:Specials Method/Manual: Permeate
Parameter 1 name P	Setpoint name (default underlined) <u>0.030</u> (0.000-10000.000)
Parameter 2 name I	Setpoint name (default underlined) <u>300.000</u> sec (0.000-10000.000)
Parameter 3 name D	Setpoint name (default underlined) <u>0.000</u> sec (0.000-10000.000)
Instruction Help text <i>These parameters are used to tune the feedback control when TMP_Control is active. For details, see "Feedback tuning" chapter in User/Instrument Manual ..</i>	

13.3.16 Flux_PI_RetentateControlValve

Instruction name Flux_PI_RetentateControlValve	Group System Settings: Specials Method/Manual: Permeate
Parameter 1 name P	Setpoint name (default underlined) <u>0.100</u> (0.000-10000.000)
Parameter 2 name I	Setpoint name (default underlined) <u>20.000</u> sec (0.000-10000.000)
Instruction Help text <i>These parameters are used to tune the feedback control when Flux_Control is active. For details, see "Feedback tuning" chapter in User/Instrument Manual ..</i>	

13.3.17 PUF_PI_RetentateControlValve

Instruction name PUF_PI_RetentateControlValve	Group System Settings: Specials Method/Manual: Permeate
Parameter 1 name P	Setpoint name (default underlined) <u>0.100</u> (0.000-10000.000)
Parameter 2 name I	Setpoint name (default underlined) <u>20.000</u> sec (0.000-10000.000)
Instruction Help text <i>These parameters are used to tune the feedback control when Permeate_Unrestricted_Flow is active. For details, see "Feedback tuning" chapter in User/Instrument Manual ..</i>	

13.3.18 PUF_PI_PermeatePump

Instruction name PUF_PI_PermeatePump	Group System Settings:Specials Method/Manual: Permeate
Parameter 1 name P	Setpoint name (default underlined) <u>0.001</u> (0.000-10000.000)
Parameter 2 name I	Setpoint name (default underlined) <u>200.000</u> sec (0.000-10000.000)
Instruction Help text <i>These parameters are used to tune the feedback control when Permeate_Unrestricted_Flow is active. For details, see "Feedback tuning" chapter in User/Instrument Manual ..</i>	

13.3.19 pNFF_PI

Instruction name pNFF_PI	Group System Settings:Specials Method/Manual: Permeate
Parameter 1 name P	Setpoint name (default underlined) <u>0.050</u> (0.000-10000.000)
Parameter 2 name I	Setpoint name (default underlined) <u>150.000</u> sec (0.000-10000.000)
Instruction Help text <i>These parameters are used to tune the feedback control when Normal_Flow_Filtration is active. For details, see "Feedback tuning" chapter in User/Instrument Manual ..</i>	

13.3.20 Fractionation

Instruction name Fractionation	Group Method/Manual: Permeate
Parameter name FracSize	Position name (default underlined) <u>0.000</u> ml (0 - 50)
Instruction Help text <i>Starts fraction collection if the fraction size specified by the FracSize parameter is >0 ml. Tube change is delayed with respect to the set delay volume. Fractionation is stopped with the instruction FractionationStop.</i>	

FeedTube

Instruction name FeedTube	Group Method/Manual: Permeate
Instruction Help text <i>During Fractionation FeedTube moves the tube rack forward one tube after the set delay volume has been collected and a fraction mark is given. When Fractionation is not used, FeedTube moves the rack instantly and no fraction mark is given.</i>	

FractionationStop

Instruction name FractionationStop	Group Method/Manual: Permeate
Instruction Help text <i>Stops fraction collection after the set delay volume has been collected.</i>	

ResetFracNumber

Instruction name ResetFracNumber	Group Method/Manual: Permeate
Warning Help text <i>ResetFracNumber is not allowed during fractionation. Instruction ignored</i>	

13.4 Transfer instructions

13.4.1 Transfer Flow

Instruction name	Group
Transfer Flow	Method/Manual: Transfer
Parameter name	Position name (default underlined)
FlowRate	<u>0.0</u> ml/min (0-200.0)
Instruction Help text	
<i>Starts the flow on the Transfer pump at the set flow rate.</i>	

13.4.2 Constant_Retentate_Volume

Instruction name	Mode	Note	Group
Constant_Retentate_Volume	Control Mode	Qt = Qp	Method/Manual: Transfer
Parameter name			Position name (Default underlined)
Mode			<u>CRV_Off</u> , CRV_On
Instruction Help text			
<i>CRV_On = liquid level in the reservoir is kept constant at the level detected when instruction activated. Instruction adjusts Transfer Flow to compensate for detected changes in level. CRV_Off = immediate inactivation of instruction. If retentate valve is open when executing Constant_Retentate_Volume a warning will be raised. Instruction ignored. If P-VB-recycle is open when executing Constant_Retentate_Volume CRV_On, a warning will be raised. Instruction ignored.</i>			
Warning Help text 1			
<i>Constant_Retentate_Volume is not allowed with Retentate_Valve_Block open. Instruction ignored.</i>			
Warning Help text 2			
<i>Constant_Retentate_Volume is not allowed with P-VB-recycle position open. Instruction ignored.</i>			

13.4.3 Transfer_Valve_Blocks

Instruction name Transfer_Valve_Blocks	Group Method/Manual:Transfer
Parameter name Macro	Position name (default underlined) <u>Closed</u> , T-VB- In1, T-VB-In2, T-VB-In3, T-VB-In4, T-VB-In5, T-VB-In6, T-VB-In7, T-VB-In8
Instruction Help text <i>Selects the position for Transfer_Valve_Blocks, T-VB- In1, T-VB-In2, T-VB-In3, T-VB-In4, T-VB-In5, T-VB-In6, T-VB-In7, T-VB-In8</i> <i>The inlet valves will not be set to their default position (closed) at End until the flow on the pump has gone down to 0 ml/min.</i>	

13.4.4 Transfer_Purge_Valve

Instruction name Transfer_Purge_Valve	Group Method/Manual:Transfer
Parameter name Instruction	Position name (default underlined) <u>To_Reservoir</u> , Waste
Instruction Help text <i>Selects transfer flow direction to Reservoir or Waste. Waste position is useful when cleaning the transfer line</i>	

13.4.5 Set_TrVol_Totalizer

Instruction name Set_TrVol_Totalizer	Group Method/Manual: Transfer
Parameter name Volume	Set point (default underlined) <u>0.00</u> ml (0.00 – 5000.00)
Instruction Help text <i>Sets a desired volume for totalizer. Transfer pump flow will increase value.</i>	

13.4.6 DF_Exchange_Factor

Instruction name DF_Exchange_Factor	Group Method/Manual: Transfer
Parameter 1 name Mode	Position name (default underlined) <u>Off</u> , On
Instruction Help text <i>DiaFiltration Exchange Factor calculates the relation between total buffer volume introduced during diafiltration step and the retentate volume when diafiltration was started.</i>	

13.4.7 MethodBase

Instruction name MethodBase	Group Method/Manual: Transfer
Parameter 1 name Base	Position name (default underlined) FeedPump, TransferPump and <u>PermeatePump</u>
Instruction Help text <i>Sets the base for method volume calculation to chosen pump.</i>	

13.4.8 ConstRVol_P

Instruction name ConstRVol_P	Group System Settings:Specials, Method/Manual: Permeate
Parameter 1 name P	Setpoint (default underlined) <u>50.000</u> mbar (0.000-10000.000)
Instruction Help text <i>Parameter affects respons of CRV algorithm. A higher value causes faster transfer flow indrease to compensate for sinking level and vice versa. Parameter also affects how fast startlevel is restored after CRV instruction is turned off. Note: A higher value amplifies noice in the Level Sensor signal and instability may occur.</i>	

13.5 Alarms, Warnings and Monitors

13.5.1 AutoZeroUV

Instruction name AutoZeroUV	Group Method/Manual: Alarms&Mon
Instruction Help text <i>Sets the relative AU to zero.</i>	

13.5.2 AveragingTime

Instruction name AveragingTimeUV	Group System:Settings:Monitors Method/Manual: Alarms&Mon
Parameter name AvTimeUV	Setpoint(default underlined) 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, <u>1.30</u> , 2.60, 5.10 and 10
Instruction Help text <i>Filters the noise in the UV signal. Averaging time is the time interval used for calculating the moving average of the absorbance signal. A long averaging time will smooth out noise efficiently, but will also distort the peaks</i>	

13.5.3 Pressure_Filter_Factor

Instruction name Pressure_Filter_Factor	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Setpoint(default underlined) <u>30</u> , (NoFilter, 2 – 100)
Instruction Help text <i>When active all pressure signals is filtered to avoid overshooting and hysteresis during pressure regulating operations, i.e. all control modes.</i>	

13.5.4 Alarm_UV

Instruction name Alarm_UV	Group System:Settings: Alarms Method/Manual: Alarms&Mon
Parameter 1 name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Parameter 2 name High Alarm	Setpoint(default underlined) <u>6000.000</u> mAU (-6000-6000)
Parameter 3 name Low Alarm	Setpoint(default underlined) <u>-6000.000</u> mAU (-6000-6000)
Instruction Help text <i>Sets the Alarm limits for the UV signal.</i>	

13.5.5 Alarm_pH

Instruction name Alarm_pH	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Parameter 2 name High Alarm	Setpoint(default underlined) <u>14.00</u> pH (0-14)
Parameter 3 name Low Alarm	Setpoint(default underlined) <u>0.00</u> pH (0-14)
Instruction Help text <i>Sets the Alarm limits for the pH signal.</i>	

13.5.6 Alarm_Cond

Instruction name Alarm_Cond	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Parameter 2 name High Alarm	Setpoint(default underlined) <u>999.99</u> mS/cm (0.00-1000.00)
Parameter 3 name Low Alarm	Setpoint(default underlined) <u>0.00</u> mS/cm (0.00-1000.00)
Instruction Help text <i>Sets the Alarm limits for the conductivity signal.</i>	

13.5.7 Alarm_FeedPress

Instruction name Alarm_FeedPress	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) Disabled, <u>Enabled</u>
Parameter 2 name HighAlarm	Setpoint(default underlined) 5.20 <u>bar</u> (0.00-5.20 bar)
Parameter 3 name LowAlarm	Setpoint(default underlined) <u>0.00</u> bar (0.00-5.20 bar)
Instruction Help text <i>Sets the alarm limits for pressure from FeedPump. When reached the system is set to Pause. An alarm: FeedPressHighAlarm / FeedPressLowAlarm will set the system to Pause.</i>	
Alarm Help text <i>HighAlarm cause: The pressure has exceeded the HighAlarm limit LowAlarm cause: The pressure has fallen below the LowAlarm limit Pressure limits are set by AlarmFeed_Press. Press Acknowledge on the error message window. Press Continue.</i>	

13.5.8 Alarm_TrPressure

Instruction name Alarm_TrPressure	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) Disabled, <u>Enabled</u>
Parameter 2 name HighAlarm	Setpoint(default underlined) 5.20 <u>bar</u> (0.00-5.20 bar)
Parameter 3 name LowAlarm	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20bar)
Instruction Help text <i>Sets the alarm limits for the pressure from Transfer Pump. When reached the system is set to Pause. Alarm: TrfPressure HighAlarm / TrfPressure LowAlarm</i> Alarm Help text <i>HighAlarm cause: The pressure has exceeded the HighAlarm limit</i> <i>LowAlarm cause: The pressure has fallen below the LowAlarm limit</i> <i>Press Acknowledge on the error message window.</i> <i>Press Continue.</i>	

13.5.9 Alarm_DeltaP

Instruction name Alarm_DeltaP	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Parameter 2 name HighAlarm	Setpoint(default underlined) 5.20 <u>bar</u> (0.00-5.20 bar)
Parameter 3 name LowAlarm	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20bar)
Instruction Help text <i>Sets the alarm limits for DeltaP. When reached the system is set to Pause. Alarm: DeltaP_HighAlarm / DeltaP_LowAlarm</i> Alarm Help text <i>HighAlarm cause: The pressure has exceeded the HighAlarm limit</i> <i>LowAlarm cause: The pressure has fallen below the LowAlarm limit</i> <i>Press Acknowledge on the error message window.</i> <i>Press Continue.</i>	

13.5.10 Alarm_TMP

Instruction name Alarm_TMP	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Parameter 2 name HighAlarm	Setpoint(default underlined) 5.20 <u>bar</u> (0.00-5.20 bar)
Parameter 3 name LowAlarm	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20bar)
Instruction Help text <i>Sets the alarm limits for TMP. When reached the system is set to Pause. Alarm: TMP_HighAlarm / TMP_LowAlarm</i> Alarm Help text <i>HighAlarm cause: The pressure has exceeded the HighAlarm limit</i> <i>LowAlarm cause: The pressure has fallen below the LowAlarm limit</i> <i>Press Acknowledge on the error message window.</i> <i>Press Continue.</i>	

13.5.11 Alarm_Flux

Instruction name Alarm_Flux	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Parameter 2 name HighAlarm	Setpoint(default underlined) <u>400</u> LMH (0-400)
Parameter 3 name LowAlarm	Setpoint(default underlined) <u>0</u> LMH (0-400)
Instruction Help text <i>Sets the alarm limits for Flux. When reached the system is set to Pause. Alarm: Flux_HighAlarm / Flux_LowAlarm</i> Alarm Help text <i>HighAlarm cause: The flow has exceeded the HighAlarm limit</i> <i>LowAlarm cause: The flow has fallen below the LowAlarm limit</i> <i>Press Acknowledge on the error message window.</i> <i>Press Continue.</i>	

13.5.12 Alarm_pNFF

Instruction name Alarm_pNFF	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Parameter 2 name HighAlarm	Setpoint(default underlined) <u>5.20</u> bar (0.00-5.20)
Parameter 3 name LowAlarm	Setpoint(default underlined) <u>0.00</u> bar (0-5.20)
Instruction Help text <i>Sets the alarm limits for pNFF. When reached the system is set to Pause. Alarm: pNFF_HighAlarm / pNFF_LowAlarm</i> Alarm Help text <i>HighAlarm cause: The pressure has exceeded the HighAlarm limit</i> <i>LowAlarm cause: The pressure has fallen below the LowAlarm limit</i> <i>Press Acknowledge on the error message window.</i> <i>Press Continue.</i>	

13.5.13 Alarm_Shear

Instruction name Alarm_Shear	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter 1 name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Parameter 2 name HighAlarm	Setpoint(default underlined) <u>20000</u> s ⁻¹ (0-20000)
Parameter 3 name LowAlarm	Setpoint(default underlined) <u>0</u> s ⁻¹ (0-20000)
Instruction Help text <i>Sets the alarm limits for Shear. When reached the system is set to Pause. Alarm: Shear _HighAlarm / Shear _LowAlarm</i> Alarm Help text <i>HighAlarm cause: The flow has exceeded the HighAlarm limit</i> <i>LowAlarm cause: The flow has fallen below the LowAlarm limit</i> <i>Press Acknowledge on the error message window.</i> <i>Press Continue.</i>	

13.5.14 All Valves, V1-V4

Instruction name Alarm_Valves	Group System Settings/Alarms Method/Manual: Alarms&Monitors
Parameter name T_VB1	Position name (default underlined) Disabled, <u>Enabled</u>
Parameter name T_VB2	Position name (default underlined) Disabled, <u>Enabled</u>
Parameter name R_VB	Position name (default underlined) Disabled, <u>Enabled</u>
Parameter name P_VB	Position name (default underlined) Disabled, <u>Enabled</u>
Instruction Help text <i>Enable/Disable the valve alarms. If a valve is not responding, an alarm is raised and the system is set to Pause. After at least 5 sec, press Continue button. If the alarm persists, try to restart the system. If the alarm still persists, check if the valve is damaged permanently. For details, see ÅKTA Instrument Handbook.</i>	
Alarm Help text <i>Valve error, valve not responding. After at least 5 sec, press Continue button. If the alarm persists, try to restart the system. If the alarm still persists, check if the valve is damaged permanently. For details, see ÅKTA Instrument Handbook.</i>	

13.5.15 Alarm_FlowPath

Instruction name Alarm_FlowPath	Group System Settings: Alarms Method/Manual: Alarms & Mon
Parameter name Mode	Position name (default underlined) <u>Enabled</u> , disabled
Instruction Help text <i>Enable/Disable the Alarm_Flow Path. The Alarm_FlowPath checks for closed flow paths (if all Inlets closed, all Outlets closed,) when Flow> 0. An alarm will be raised and the System is set to Pause.</i>	
Alarm1 Help text <i>TrfFlow> 0 and inlets are closed</i>	
Alarm2 Help text <i>PermFlow> 0 and outlets is closed.</i>	

13.5.16 Alarm_Airsensor

Instruction name Alarm_AirSensor	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Instruction Help text <i>Sets the alarm for air sensor. An alarm will set the system to Pause if air is detected in air sensor flow cell.</i>	

13.5.17 Alarm_ZeroLevel

Instruction name Alarm_ZeroLevel	Group System:Settings: Alarms Method/Manual:Alarms&Monitors
Parameter name Mode	Position name (default underlined) <u>Disabled</u> , Enabled
Instruction Help text <i>Sets the alarm for ZeroLevel. An alarm will set the system to Pause if zero level is indicated by the level sensor.</i>	

13.5.18 WatchPar_UV

Instruction name WatchPar_UV	Group System:Settings:Monitors Method/Manual: Alarms&Mon
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> mAU (0-6000)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> mAU (0-6000)
Instruction Help text <i>Watch on UV signal. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction (see UNICORN User Manual). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.19 WatchPar_pH

Instruction name WatchPar_pH	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter name DeltaPeak	Setpoint(default underlined) <u>0.00</u> pH (0-14)
Parameter name Delta_Base	Setpoint(default underlined) <u>0.00</u> pH (0-14)
Instruction Help text <i>Watch on pH signal. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.20 WatchPar_Cond

Instruction name WatchPar_Cond	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> mS/cm (0-999.9)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> mS/cm (0-999.9)
Instruction Help text <i>Watch on conductivity signal. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition for the Watch instruction</i>	

13.5.21 WatchPar_FeedPress

Instruction name WatchPar_FeedPress	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Instruction Help text <i>Watch on pressure reported at filter feed port. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.22 WatchPar_RetenPress

Instruction name WatchPar_RetenPress	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Instruction Help text <i>Watch on pressure at filter retentate port. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual.). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.23 WatchPar_PermPress

Instruction name WatchPar_PermPress	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Instruction Help text <i>Watch on pressure at permeate filter port. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.24 WatchPar_FeedFlow

Instruction name WatchPar_FeedFlow	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.0</u> ml/min (0.0-600.0)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.0</u> ml/min (0.0-600.0)
Instruction Help text <i>Watch on flow reported from feed pump. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual.). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.25 WatchPar_RetFlow

Instruction name WatchPar_RetFlow	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00 ml/min</u> (0.00-600 ml/min)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00 ml/min</u> (0.00-600 ml/min)
Instruction Help text <i>Watch on flow reported for retentate flow. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual.). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.26 WatchPar_PermFlow

Instruction name WatchPar_PermFlow	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.0 ml/min</u> (0.0-200.0 ml/min)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.0 ml/min</u> (0.0-200.0 ml/min)
Instruction Help text <i>Watch on flow reported from permeate pump. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual.). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.27 WatchPar_TrffFlow

Instruction name WatchPar_TrffFlow	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.0</u> ml/min (0-200)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.0</u> ml/min (0-200)
Instruction Help text <i>Watch on flow reported from transfer pump. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual.). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.28 WatchPar_RetVol

Instruction name WatchPar_RetVol	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> ml (0.00 – 80000.00)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> ml (0.00 – 80000.00)
Instruction Help text <i>Watch on retentate volume. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.29 WatchPar_ResVol

Instruction name WatchPar_ResVol	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> ml (0.00 – 80000.00)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> ml (0.00 – 80000.00)
Instruction Help text <i>Watch on reservoir volume. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.30 WatchPar_PermVol

Instruction name WatchPar_PermVol	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> ml (0.00 – 80000.00)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> ml (0.00 – 80000.00)
Instruction Help text <i>Watch on permeate volume. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.31 WatchPar_TransVol

Instruction name WatchPar_TransVol	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> ml (0.00 – 80000.00)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> ml (0.00 – 80000.00)
Instruction Help text <i>Watch on transfer volume. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.32 WatchPar_DeltaP

Instruction name WatchPar_DeltaP	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Instruction Help text <i>Watch on DeltaP reported. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.33 WatchPar_TMP

Instruction name WatchPar_TMP	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00 bar</u> (0.00-5.20 bar)
Instruction Help text <i>Watch on TMP reported. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.34 WatchPar_Flux

Instruction name WatchPar_Flux	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.0 LMH</u> (0.0-120000.0 LMH)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.0 LMH</u> (0.0-120000.0 LMH)
Instruction Help text <i>For Watch on flux reported from permeate pump. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual.). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.35 WatchPar_Shear

Instruction name WatchPar_Shear	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0</u> s ⁻¹ (0-20000)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0</u> s ⁻¹ (0-20000)
Instruction Help text <i>For Watch on Shear reported from feed pump. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual.). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.36 WatchPar_pNFF

Instruction name WatchPar_pNFF	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> bar (0.00-5.20)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> bar (0.00-5.20)
Instruction Help text <i>For Watch on pNFF. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.37 WatchPar_%_FLUX_Drop

Instruction name WatchPar_%FluxDrop	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.0%</u> (0.0 – 100.0)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.0%</u> (0.0 – 100.0)
Instruction Help text <i>Watch on %_Flux_Drop reported.The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.38 WatchPar_ConcFactor

Instruction name WatchPar_ConcFactor	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> (0.00 -50.00)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> (0.00 -50.00)
Instruction Help text <i>Watch on concentration factor reported.The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual.). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.5.39 WatchPar_DF_X_Fact

Instruction name WatchPar_DF_X_Fact	Group System:Settings: Monitors Method/Manual:Alarms&Monitors
Parameter 1 name DeltaPeak	Setpoint(default underlined) <u>0.00</u> (0.00 -50.00)
Parameter 2 name Delta_Base	Setpoint(default underlined) <u>0.00</u> (0.00 -50.00)
Instruction Help text <i>Watch on DF_X_aFct reported. The DeltaPeak setting affects only the Less_than_or_valley and Peak_max_conditions when using the Watch instruction, see UNICORN user manual. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Delta_Base setting affects only the Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the Delta_Base value up or down over the time interval specified in the Stable_baseline condition in the Watch instruction.</i>	

13.6 Fraction collector instructions

FracParameters

Instruction name FracParameters	Group System:Settings:Specials
Parameter 1 name DelayVol	Setpoint(default underlined) <u>0.000</u> ml (0.000-10.000)
Parameter 2 name TubeChange	Setpoint(default underlined) <u>Tube</u> , DropSync, WasteBetweenTubes
Instruction Help text <i>Parameter settings for fraction collector. Delay volume is defined as the volume from the UV cell to the end of the tubing for the fraction collector. Collection of the flow during tube change can be handled in different ways. Tube: No synchronisation of collection. DropSync: Tube change synchronised to drop release. Should only be used at flow rates generating drops. WasteBetweenTubes: The flow will be diverted to waste when moving between tubes.</i>	

FracNumberingMode

Instruction name FracNumberingMode	Group System_Settings:Specials
Parameter name Mode	Position name (default underlined) <u>Reset</u> , Continue
Instruction Help text <i>Determines whether fraction number is reset at the end of a method or not. Reset sets Tube No to 1 after method end. Continue continues numbering from the last tube in the previous method.</i>	

13.7 UPC

13.7.1 UV Monitor

UVLampOFF

Instruction name UVLampOFF	Group Method/Manual: Alarms&Mon
Instruction Help text <i>Sets the UV lamp permanently to OFF. To start UV lamp system and computer has to be re-booted.</i>	

13.7.2 pH

pHTempComp

Instruction name pHTempComp	Group System:Settings: Monitors
Parameter name pHTempComp	Position name (default underlined) <u>OFF</u> , ON
Instruction Help text <i>Sets the temperature compensation on or off. For more accurate measurements during temperature changes, the pH measurement can be temperature compensated. When using pHTempComp it is important that the temperature of the pH electrode is the same as that of the conductivity flow cell since that is where the temperature is measured.</i>	

13.7.3 Cond

CondTempComp

Instruction name CondTempComp	Group System:Settings: Monitors
Parameter name CompFactor	Setpoint(default underlined) <u>2.0</u> % (0.0-9.9)
Instruction Help text <i>Relates conductivity to temperature. The compensation consists of a compensation factor together with a reference temperature (CondRefTemp). All conductivity values will then automatically be converted to the set reference temperature. The factor is expressed in percentage increase of conductivity per °C increase in temperature. If the CompFactor is unknown, a general approximate value of 2% can be set for many common salt buffers. 0% = off.</i>	

CondRefTemp

Instruction name	Group
CondRefTemp	System:Settings: Monitors
Parameter name	Setpoint(default underlined)
RefTemp	<u>25.0</u> °C (0.0-99.9)
Instruction Help text	
<i>Sets the reference temperature to which the measured conductivity values will be converted. CondRefTemp is active when a factor of CondTempComp is selected.</i>	

13.8 Watch

These signals should have a Watch instruction (defined by UNICORN) in the following order and also be possible to select Watch_Off for:

Hold_Until, UV, pH, Cond, Feed_Press, Reten_Press, FeedFlow, R_Flow, Perm_Flow, Trans_Flow, RetVol, PermVol, TransVol, DeltaP, TMP, Flux; Shear, ConcFactoror, DF_X_Fact, FluxDrop, Airtensor, ZeroLevel, AuxIn1, AuxIn2, AuxIn3, AuxIn4

These signals should be possible to select Hold_Until for (defined by UNICORN) in the following order:

UV, pH, Cond, Feed_Press, Reten_Press, FeedFlow, R_Flow, Perm_Flow, Trans_Flow, RetVol, PermVol, TransVol, DeltaP, TMP, Flux; Shear, ConcFactoror, DF_X_Fact, FluxDrop, Airtensor, ZeroLevel, AuxIn1, AuxIn2, AuxIn3, AuxIn4

13.9 Calibration

13.9.1 ZeroLS

Monitor ZeroLS	Function 0-calibration of level sensor
Text 1 0-calibrate the level sensor. See Help.	

13.9.2 LevelsensorZeroCalibration

Instruction name LevelsensorZeroCalibration	Group System:Settings: Monitors
Instruction Help text <i>Give user access to Calibration instruction in order to make it possible to calibrate Level sensor from a method</i>	

13.9.3 UPC

pH

Monitor pH	Function Calibration of pH electrode
Text 1 and 2 Calibrate pH electrode for buffer 1. See Help. Calibrate pH electrode for buffer 2. See Help.	
Parameter 1 name Reference value 1	Input (default underlined) <u>0.00</u> pH (0.00-14.00)
Parameter 2 name Reference value 2	Input (default underlined) <u>0.00</u> pH (0.00-14.00)
Result Calibrated electrode slope	Value 1 0.0 %
Result Asymmetry potential at pH 7	Value 2 0.0 mV
Help text <i>The pH Monitor is calibrated using standard buffer solutions in a two point calibration. Difference between the buffers should be at least 1 pH unit. A new electrode has a slope of, typically, 95-102% and an asymmetry potential within ± 30mV. As a rule, when an electrode has an asymmetry potential outside of ± 60mV and a slope lower than 80%, and no improvements can be achieved by cleaning, it should be replaced.</i>	

Cond_Calib

Monitor Cond_Calib	Function Calibration of cell constant
Text 1 Determination of cell constant for Cond cell. See Help.	
Parameter name Reference value 1	Input (default underlined) <u>1.000</u> mS/cm (1.0-999.9)
Result Cell constant	Value 1/cm (0.1-300.0)
Help text <i>Calibration of Cond, conductivity cell. Normally it is not necessary to adjust the cell constant as the flow cell is pre-calibrated on delivery. Set CondTempComp to 0 in System: Settings prior calibration of cell constant. The temperature sensor must be calibrated before adjusting the cell constant. Fill the flow cell with calibration solution of 1.00 M NaCl. Wait until the temperature is constant in the range 20-30 °C. Enter the theoretical conductivity value according to graph in Instrument Handbook, Basic operation, Calibration</i>	

Cond_Cell

Monitor Cond_Cell	Function Enter a new cell constant
Text 1 Add cell constant value of a new Cond cell. See Help	
Parameter name Reference value	Input (default underlined) <u>40.0</u> 1/cm (0.1-300.0)
Help text <i>To be performed when you are replacing the current cell with a new conductivity cell. The cell constant is shown on the packaging. In case you have thrown the outer package, perform Cond_Calib.</i>	

Cond_Temp

Monitor Cond_Temp	Function Temperature sensor
Text 1 Calibrate the Cond temperature sensor. See Help.	
Parameter name	Input (default underlined)
Reference value	<u>0.0</u> °C (-5.0-60.0)
Help text <i>Calibration of the temperature sensor in the conductivity flow cell is only necessary if the monitor is used in high accuracy measurements. Place the Cond flow cell together with a precision thermometer inside a box or empty beaker to ensure that they are not exposed to draft. Leave them for 15 min to let the temperature stabilise. Read the temperature on the thermometer and enter this as Reference value.</i>	

TrfPress

Monitor TrfPress	Function Pressure calibration
Text 1 Sets the pressure reading to zero. See Help.	
Help text <i>Calibrate pressure offset according to step 1: Calibrate pressure offset for Transfer pump. Click the Start calibrate button. For details see ÄKTA Instrument Handbook.</i>	



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